ELSEVIER

Journal of Chromatography B, 771 (2002) 289–301

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Protein mapping in rat basophilic leukaemia cells

K.M. Carroll*, E.M. Carey, B.A. Helm

Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield S10 2UH, UK

Abstract

This review discusses some of our recent studies on rat basophilic leukaemia (RBL) cells, a model cell line for mast cell function. Our interest in these cells is a consequence of the role played by mast cells in the allergic response. Thus far we have described the identification of several spots, and their pI/M_r co-ordinates. Here we describe how we can further decipher the mast cell proteome using a variety of staining/immuno-blotting procedures. We demonstrate the sensitivity of staining procedures and immuno-blotting using an anti-phosphotyrosine antibody. Our aim is to contribute to the ever-expanding two-dimensional gel and phosphoprotein databases currently available. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Protein mapping; Rat basophilic leukaemia cells; Proteomics

Contents

1. Introduction 289
2. Protein maps created by two-dimensional electrophoresis 290
3. Rat basophilic leukaemic (RBL) cells as a model of mast cell function 291
4. Functional proteomics applied to RBL cell activation 292
5. Databases and 2-D maps 298
6. Databases and software 299
7. Future perspectives 299
   7.1. Cell specific reference gels 299
   7.2. Analysis of human mast cell proteomes 300
8. Nomenclature 300
Acknowledgements 300
References 300

1. Introduction

In any given organism, gene expression is regulated in a cell specific fashion and controls diverse cell functions, such as cell proliferation, differentiation and cell responses to external stimuli. A proteomics based approach to investigating cell function focuses on the identification of key proteins characteristic of a given cell's phenotype, in particular those proteins, which control and exhibit responses to defined environmental stimuli. The

*Corresponding author. Fax: +44-114-2728-697.
E-mail address: k.carroll@sheffield.ac.uk (K.M. Carroll).

1570-0232/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1570-0232(02)00111-3

protein composition or proteome of a particular cell/tissue is never static but reflects an adaptation to signals, which induce or repress specific genes. Functional proteomics is the determination of the role of an individual protein or group of proteins linked in some way, as in a signalling pathway or by a common post-translational activation/inhibition mechanism, within the context of the entire cell proteome. Proteomics therefore assesses alterations in protein expression and post-translational modifications associated with developmental and disease states, as well as those occurring in response to environmental stimuli. A major step forward in proteomics has been the application of mass spectrometry (MS) that provides a rapid and sensitive means of protein characterisation. A proteomics based approach, analysing protein function within the context of the entire cell proteome (a holistic approach), can address questions concerning the role of individual proteins or a group/cassette of functionally related proteins, their differential expression in health and disease states, responses to external stimuli or functions of protein isoforms arising from genetic polymorphisms. This opens up new possibilities for the discovery of novel targets for therapeutic interventions, within a wider context than previously possible. With the availability of genomic DNA sequences from databases that emerged as a result of the genome project, the combination of proteomics and genomics facilitates the rapid identification of encoded and expressed proteins from many organisms, changes in expression levels and provides a context of function within the entire proteome.

This review presents some of our recent work using a proteomics based approach to analyse protein expression in cells of mast cell lineage, employing two-dimensional electrophoresis (2-DE) methodology for protein resolution followed by staining of proteins using a variety of techniques.

2. Protein maps created by two-dimensional electrophoresis

The resolution of complex mixtures of proteins such as whole cell extracts by a combination of 2-DE with first-dimensional resolution a function of isoelectric point (pI) and second-dimensional resolution on polyacrylamide gels (PAGE) a function of molecular mass (M_r), should generate a protein map of the entire cell protein. The characterisation of individual protein ''spots'' by highly sensitive MS analytical techniques has opened up the possibility of identifying all proteins expressed by a particular organism or cell type.

Protein resolution of complex mixtures, usually from whole cell extracts, is the starting point of proteomics. Most laboratories use the 2-DE–PAGE procedure, which emerged from pioneering studies in the last century. The co-ordinates of M_r/pI for each peptide in the two-dimensional (2-D) map provide an initial characterisation, with definitive identification achieved by subsequent recovery of protein from the gel and identification by MS.

Various 2-D procedures have been applied to the resolution of proteins in complex mixtures in which resolution of proteins in each dimension is a function of one particular characteristic (surface charge, size, and hydrophobicity). Compared with techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) alone or in tandem, with their requirement for sophisticated equipment to achieve optimum resolution, 2-DE–PAGE is a relatively straightforward procedure. Advantages of this approach are that the equipment is relatively simple, and in essence an extension of the one dimensional (1-D) PAGE separation of polypeptides and for which standard equipment is commercially available, although 2-DE offers much enhanced resolution. Improved reproducibility in the first dimension has been achieved through the development of immobilised pH gradients with ampholytes covalently attached to matrix supports (IPG gels), which are now commercially available. There has also been the development of several highly sensitive stains for the detection of proteins over a wide dynamic range, covering nano-, pico- and femtomolar amounts of protein, appropriate for N-terminal sequencing and MS analysis. 2-DE is compatible with analytical and semi-preparative procedures, and wide variations in sample size from 50 to 500 μg protein or higher can be applied, with usually only a slight reduction in resolution at high sample loads.

Successful generation of reproducible 2-D maps

requires a certain degree of manual dexterity. Reproducibility is essential. It can be affected by a number of factors, including sample preparation (the extraction of strongly hydrophobic proteins may be incomplete), unintentional variations in the precise protocol, the composition and storage/ageing of protein solubilisation buffers and gels, purity of reagents, cleanliness of equipment, stability of high voltage in first dimension.

Currently, there are three main systems commercially available that have been developed for routine two-dimensional electrophoresis, which rely on immobilised pH gradients. These three systems are Multiphor II, IPGPhor (Amersham Pharmacia Biotech), and the Protean IEF. All three have been shown to give a high degree of quantitative reproducibility in one independent test [1]. The system used in our laboratory utilises the Multiphor II for first dimension IEF and Bio-Rad Protean IIxi for second dimension.

3. Rat basophilic leukaemic (RBL) cells as a model of mast cell function

Techniques have been described for the generation of human mast cells from pluripotent progenitor cells [2]. However their limited availability, and also that of primary mast cells obtained from human tissue, is a serious limitation to their routine use. The fact that primary mast cells cannot be maintained in culture for extended periods, together with the expense associated with the purification of human mast cells and basophils, is a potential difficulty. The absence of a suitable functional human mast cell line—the HMC-1 line only responds poorly when stimulated through FcεRI [3]—make the rat basophilic leukaemia cell line, RBL-2H3, an attractive model system to study the consequences of FcεR1-mediated cell activation and concurrent changes in protein expression/modification. This cell line arose as a result of chemical induction of basophilic leukaemia in rats and following the transplantation of the basophil tumour a variant termed RBL-2H3, which has an immature mast cell-like phenotype, was cloned, which responded with high levels of mediator release following IgE-mediated receptor cross-linking. Widespread use and subcloning of this cell line has resulted in genetic variants where the secretory potential has changed [4,5]. Our studies thus far have concentrated on RBL cells lines selected on the basis of high- and low-secreting phenotypes. It has to be recognised that a transformed cell line is likely to have major differences in signalling pathways compared with primary mast cells. Nevertheless, aspects of mast cell function can be examined using RBL cells, and the same approach can subsequently be used for the construction of protein maps and analyses of protein function in primary human mast cells and basophils.

The interest in mast cell and basophil biochemistry/physiology is a direct consequence of the central role of these cells in the allergic response [2]. These cells express high affinity receptors (FcεRI) for immunoglobulin (Ig)E and following activation of IgE receptor linked pathways, secrete powerful pharmacologically active mediators [2]. The consequences of IgE-mediated degranulation are best known for their pathological role in allergic disease and asthma, although there is clear evidence for their involvement in important host defence mechanisms against some parasite and bacterial infections [6,7]. Rapid responses to an FcεRI-mediated stimulus occur within 60 s. During this period, reversible changes in protein phosphorylation and lipidation, affecting several proteins have been demonstrated [8]. Subsequent downstream signalling activates partially characterised diverse pathways which link the receptor-mediated stimulus to the rapid release of preformed inflammatory mediators stored in granules including histamine and leukotrienes, and the increased synthesis and release of de novo synthesised mediators, including important immune modulatory molecules, such as pro-allergenic cytokines and chemokines. The induction of these proteins has been reported to occur within 1–3 h after cell activation [9,10].

Over the past decade changes in gene expression in IgE activated mast cell lines have been pursued in many laboratories by a variety of methods, including Northern blotting [11], subtraction libraries [12], reverse transcription polymerase chain reaction (RT-PCR) [13], and SAGE (serial analysis of gene expression) [14]. The identification of a small number ($<1\%$) of differentially expressed genes, usually assessed 3 h after activation, has been observed [14].

Quantitative analysis of the levels of transcription of differentially expressed genes has however proven difficult [14].

4. Functional proteomics applied to RBL cell activation

Our long-term aims in adopting a functional proteomics approach to analysing cellular responses to cell activation and degranulation via IgE-mediated and IgE-independent pathways are to identify unique, cell-specific proteins regulating mast cell responses, in particular those controlling the early and late stages leading to mediator secretion. This includes events involving specific receptors, e.g., FcεRI-mediated degranulation, activation via the c-kit receptor or, e.g., mast cell responses to mediators released from other effector cells of the immune system like eosinophils and platelets during the late phases of allergic responses [15]. Furthermore, we and others have shown that proteases, phospholipases and lectins associated with sources of environmental allergens can activate cells of mast cell/basophilic lineage in the absence of sensitisation with allergens specific IgE [9,10]. As drug therapies become increasingly targeted to cell specific proteins and processes, a functional proteomics approach can therefore be expected to provide an overall view of mast cell/basophil responses to diverse receptor-mediated and environmental stimuli. Its goal is to identify key polypeptides controlling different stages of the cell's response to allergens.

A proteomics based approach should provide information on unique ligand/receptor mediated and cell-specific signalling pathways. It should identify common elements in pathways controlling, e.g., Ca^{2+} influx and mobilisation, protein lipidation and phosphorylation status through activation of protein kinases and phosphatases, since many diverse stimuli affecting cells of mast cell/basophil lineage culminate in regulated exocytosis and activation of gene transcription [10]. We may obtain answers to questions regarding the balance between different pathways, the strength of signal required for activation and its duration, all of which are known to affect cell responses. Mast cell specific targets may become apparent and open up the possibility of investigating specific protein–protein interaction as potential targets for therapeutic intervention.

Covalent modifications of existing proteins including serine, threonine and tyrosine phosphorylation and dephosphorylation [5,8], nucleotide binding [16], and lipidation [8], are known to occur in RBL and mast cells. They are of critical importance both in the early stages of FcεRI activation, leading to the recruitment of cytoplasmic protein kinases to the receptor (phosphorylation, lipidation) through to the terminal stages of exocytosis, which involves monomeric G-proteins of the Ras related Rab3 family controlling fusion of secretory granules with the plasma membrane [16]. In mast cells, the interplay of these components governs the temporal sequence of events and biochemical/physical interactions of proteins operating in downstream signalling connecting receptor aggregation to degranulation and to activation of mast cell gene expression.

There have been few studies investigating the mast cell and RBL cell proteome. Kouretas et al., more than a decade ago, compared primary rat peritoneal mast cells and RBLs [17]. The 2-D gel patterns were reported to be highly reproducible and over a pH range of 3.5–8.5 some 200 ''spots'' were detected by silver staining. Fewer ''spots'' were detected in cells labelled with [^{35}S]cysteine. As observed in many studies, basic proteins were largely undetected or unresolved. These results were obtained with RBLs, which had been removed from tissue culture dishes by trypsin, which is now known to induce mast cell degranulation by an unidentified mechanism [10]. Also a carrier ampholyte system/tube gel was used, now superseded by the development of IPG gels.

As described in an earlier publication [18], our starting point in using 2-D PAGE analysis to map the RBL cell proteome required the construction of an average 2-D map, which required reproducible protein maps of whole cell extracts, with defined landmark proteins. It became apparent that the combination of restricted sample capacity and detection limits inherent in spot visualisation procedures could prohibit the detection of both low abundance proteins and hydrophobic proteins [19]. We therefore investigated the sensitivity of different staining procedures, which is important when relatively small amounts of protein are available for analysis. The quantitation and reproducibility of the

number of polypeptide spots which could be detected in 2-D gels and whose M_r/pI index could be confirmed by reproducible patterns of peptide spots was of high priority. Results shown in Table 1 compare the number of peptide spots detected in extracts of RBL-2H3.1 cells using different protein staining procedures and subsequent image capture by CCD (FluorChem 8800; Alpha Innotech, San Leandro, CA, USA) and analysis using 2-D image analysis software (Phoretix 2D Advanced; Non-Linear Dynamics, Newcastle, UK). Previously, we obtained seemingly adequate 2-D protein arrays for proteome analysis for RBL cells by applying a sample load of up to 500 μg protein solubilised from 10^6–10^7 cells, i.e., whole cell extracts [18]. A glutaraldehyde–ammoniacal silver staining protocol routinely used in our laboratory has proven sensitive; more than 1000 protein spots can be detected (Fig. 1). When development is allowed to continue until low abundance proteins appear in the gel, it becomes clear that this visualisation procedure is the most sensitive. For high abundance proteins, the gel appears to be overloaded, but the image capture system has a fourfold linear dynamic range, which enables quantitation of both low and high abundance proteins. The drawback of this protocol is that it is not compatible with subsequent DE-MALDI analysis. A modified silver staining method described by Shevshenko et al. [20], using silver nitrate and thiosulfate rather than glutaraldehyde as enhancer (Fig. 2) that is compatible with subsequent MS analysis, has been reported to have reduced sensitivity (in our hands similar detection limits to colloidal Coomassie Blue, Fig. 3). We found a higher sensitivity with Sypro Ruby stain (Fig. 4), and both colloidal Coomassie Blue and Sypro Ruby stains gave good reproducibility and evenness of staining. Both stains are easy to use, and more importantly, the visualisation procedures are compatible with subsequent MS analysis. These findings are summarised in Table 1. We are currently investigating the possibility of using Sypro Ruby visualisation of proteins transferred to poly(vinylidene fluoride) (PVDF)/nitrocellulose, in conjunction with immunodetection. These comparisons illustrate that the choice of protein stain is governed by the additional information sought after spot visualisation. Increasing the amount of protein on the gel may overcome problems associated with low sensitivity, but for protein extracted from whole cells, DNA in the sample causes increased viscosity. This can be overcome by prior treatment with DNAase or by sample sonication followed by trichloroacetic acid (TCA) precipitation and resolubilisation in sample buffer [18].

An important consideration is the reproducibility of stained gels, which can be affected by a number of factors in first and second dimension resolution such as sample preparation, storage, age of buffers and gels, variable running conditions during electrophoresis. Repetitive 2-DE analysis is required to ascertain reproducibility, as demonstrated here by comparing patterns of peptide ''spots'' in gels subjected to identical or different staining procedures. All staining methods detect high abundance polypeptides. Some of these ''spots'' were also detected with an antibody to a protein tyrosine motif (TAM) shown in Fig. 5. Surprisingly, the expression levels of several TAM containing proteins is below the detection limit for silver staining. The sensitivity of this method therefore holds considerable promise for the identification of phosphorylated proteins in resting and activated mast cells, and the temporal changes in the overall patterns can be expected to provide important information regarding the activa-

Table 1
Quantitation of ''spots'' detected with Phoretix 2D Advanced software

Staining procedure	Number of spots detected
Ammoniacal silver	1172
Sypro Ruby	669
Colloidal Coomassie	377
Shevshenko modified silver	371
Immunodetection anti-P~Y antibody	134

The table shows the number of spots obtained on 2-D gels (Figs. 1–5) visualised with different staining/blotting procedures as indicated, from cells prepared for a typical 2-DE experiment. Cells were cultured and harvested, and divided such that 3.5×10^6 cells were applied to each IEF strip. Following the second dimension run, the resulting gels were scanned and analysed using 2D Advanced software (Phoretix, Non-Linear Dynamics, Newcastle, UK). We have described the identification of proteins from Coomassie stained 2-D gels in a previous report [18]. We have yet to confirm the identity of the spots from the above gels (Figs. 1–5). For details of protein separation and staining see legends to Figs 1–5.

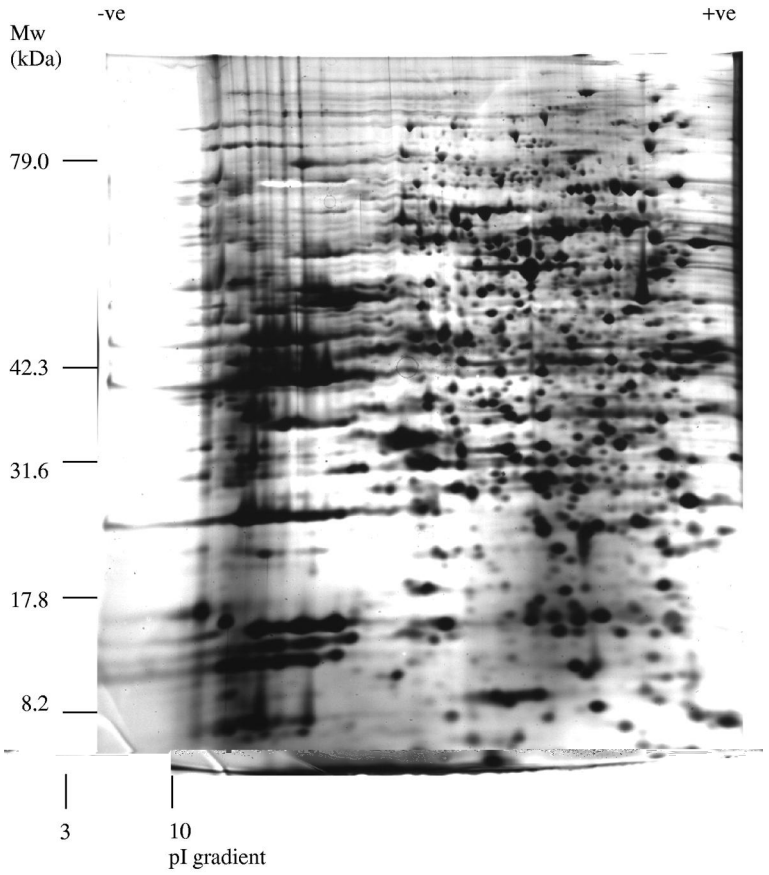

Fig. 1. Comparison of protein staining procedures for the detection of whole cell proteins from RBL cells on 2-D gels. The figure represents a peptide map obtained for the high-secreting RBL-2H3.1 variant obtained using ammoniacal silver with glutaraldehyde fixation. Cells were cultured in DME media, harvested in cell dissociation solution and washed in PBS. Whole cells ($\sim 3.5 \cdot 10^6$) were lysed and protein solubilised in an urea/CHAPS/DTT/ampholyte buffer before loading onto rehydrated IPG gels. The running conditions for electrophoresis in the first and second dimensions have been described elsewhere [18]. For comparison, the same sample load was used for each staining procedure.

tion of protein phosphatases and kinases in mast cells.

Quantitative analysis of ''spots'' of interest relative to actin or other house keeping proteins suggests that ''spot'' intensity is linear with respect to protein concentration (10 fmol–1 pmol), although this awaits verification by other methods such as ICAT [21]. However, a major and largely unresolved problem concerning 2-D gel analysis is that not all proteins will be represented in the protein map [22]. Although proteins expressed at very low abundance (<10 fmol) can be detected using image capture CCD cameras and sensitive staining procedure, which includes chemiluminescence, phosphoimaging, auto- and fluorography in the case of covalently modified or radiolabelled proteins [23], there is a real concern that large and hydrophobic proteins may not be extracted into the most commonly used urea/detergent buffers compatible with first dimension IPG gels [19]. To address this problem, it is important to examine the effect of different buffer systems on the representation of proteins in 2-D maps, to be certain of including membrane proteins in the analysis.

If required, higher resolution 2-D maps can be obtained using narrow pH range IPG gels in the first

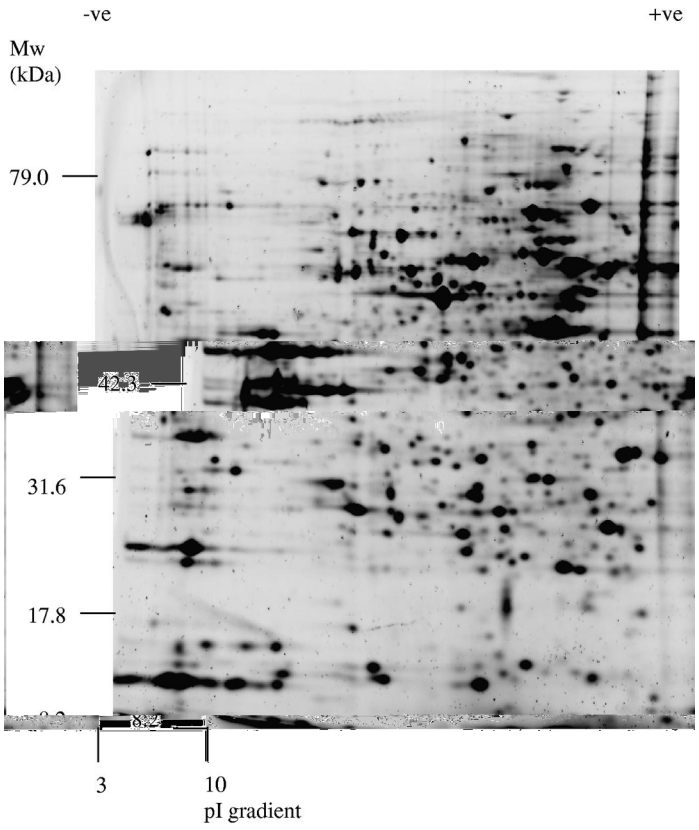

Fig. 4. Comparison of protein staining procedures for the detection of whole cell proteins from RBL cells on 2-D gels. The figure represents a peptide map obtained for the high-secreting RBL-2H3.1 variant obtained using Sypro Ruby fluorescence (negative image). Cells were cultured in DME media, harvested in cell dissociation solution and washed in PBS. Whole cells (~$3.5 \cdot 10^6$) were lysed and protein solubilised in an urea/CHAPS/DTT/ampholyte buffer before loading onto rehydrated IPG gels. The running conditions for electrophoresis in the first and second dimensions have been described elsewhere [18]. For comparison, the same sample load was used for each staining procedure.

mutase in the low secretor, reflecting differences in energy provision, which may affect cell signalling via nucleotide phosphate provision (ATP, GTP). Other proteins with diverse functions also show differential expression and may influence secretory phenotype. Prohibitin, which is over-expressed in the low-secretor can exert inhibitory effects on raf/MAPK effector pathways. Vimentin, a filament protein of the cell cytoskeleton, is present in low secretory RBL cells and has also been reported to be expressed in precursor mast cells, but normally absent from differentiated cells. Transitional ATPase (TER-ATPase) of the endoplasmic reticulum is evident in the low secretors, though its function is uncertain. Identification of other differentially expressed proteins in the RBL 2-D maps is ongoing, but the diversity of differentially expressed proteins already identified clearly illustrates the need to integrate information concerning the cellular function of these proteins into RBL and mast cells function.

There is increasing evidence that the Rab3 family of monomeric GTP binding proteins (which have effector functions determined by GTP/GDP exchange) regulate exocytosis/degranulation by controlling recruitment and reorganisation of proteins comprising the fusion complex formed between granule and cell plasma membrane. Rab proteins from a variety of cell types have similar electro-

Swiss 2-D PAGE (www.expasy.ch/cgi-bin/map), currently containing various 2-D profiles of rat tissues, e.g., liver, kidney, promyelocytic leukaemia cell etc, and some human tissues/cells e.g., plasma, platelet, red blood cell, and the bacterium *Escherichia coli* peptide map data. With the development of these databases it is now possible to obtain predictions of protein identities based on M_r/p*I* indices. While databases in their current form are incomplete, species and cell specific comprehensive databases are envisaged, including The Human Protein Index [28], although this may not be available for several years. Since there is evidence that homologous proteins from a variety of cell types from the same or different species have similar electrophoretic mobilities in 2-D arrays, M_r/p*I* data bases, which contain both actual and theoretic values, may ultimately enable the identification of peptides with some degree of certainty to be made directly from 2-D proteome maps, including the nature of post-translational modifications.

6. Databases and software

Recent advances in image analysis and ''spot'' picking tools for MS are supported by a variety of software packages, including Tycho, Melanie III, PDQuest and Phoretix 2D Advanced. The latter system is used in our laboratory and was developed by Non-Linear Dynamics, Newcastle, UK. The software facilitates molecular mass calibration and spot matching using overlay warping seeds. This procedure assists the reproducible identification of distinct ''spots'' and quantitative ''spot'' analysis; it allows background subtraction and should expedite the development of average reference gels with respect to the identification of ''landmark spots'' and the creation of gel difference maps. Another programme, Melanie III, compares M_r/p*I* protein indices and peptide mass fingerprinting from MS for mass differences between empirical and theoretical peptide masses. Some 29 different rules, which are derived from an extensive examination of post-translational modification documented in the SWISS-PROT database, are applied to peptide mass differences corresponding to known post-translational modifications [29]. However, in view of the novelty of this approach, the chemical modification assigned on the basis of computational analysis will have to be verified by chemical and biochemical analysis.

The use of 2-D gel databases in the public domain, e.g., SWISS-PROT 2-D gel database, can assist our studies by providing early clues as to the identity of proteins by comparison of M_r/p*I* co-ordinates. Since there is evidence that homologous proteins from a variety of cells and species have similar electrophoretic mobilities in 2-D arrays, databases like the Human Protein Index can be expected to expedite the identification of proteins in different cell types, subjected, for example, to different stimuli, or in mast cells and basophils from different species [2]. This is part of an ongoing worldwide effort to establish comprehensive databases with co-ordinates for all proteins expressed in a cell and species-specific manner. The phosphoprotein database gives overall patterns of changes in phosphorylation, although at the present time provides the identity of only a limited all number of phosphoproteins.

7. Future perspectives

7.1. Cell specific reference gels

The generation of reproducible and reliable reference gels is a pre-requisite for the subsequent study of mast cells activated by different stimuli and/or following different time intervals. As mentioned above, in addition to an IgE-receptor mediated stimulus, exocytosis in cells of mast cell lineage occurs in response to a multitude of different signals mediated by IgG, growth factors (e.g., c-kit ligand), Complement and chemokine receptors, exposure to eosinophil and platelet derived mediators, hydrolytic enzymes including proteases and phospholipases, and lectins [9,10]. Differential expression analysis has recently been developed and depends on modified cyanine dyes, which allow the simultaneous running of cell proteomes obtained under three different conditions and are said to increase throughput and precision [23]. This is equivalent to performing analyses on a single gel rather than single analyses on multiple gels, with obvious advantages.

7.2. Analysis of human mast cell proteomes

The production of a reference gel for a prototypic model cell line like the RBL variants assists the standardisation of methods for the study of primary human mast cells from different tissue locations (lung, skin, peritoneal cavity, stem cell derived). Established immuno-magnetic methods are applied in the isolation and purification of human lung mast cells (HLMCs), and a one-step purification to 97% homogeneity is possible following physical and enzymatic dispersion of human lung tissue following lung resection (P.T. Peachell and K. Carroll, unpublished). Single preparations can yield in excess of 10 million HLMCs. The degree of cell purity and cell number therefore make it possible to carry out a number of investigations on primary resting and ligand activated HLMCs with cells from a single individual. It is however likely, that comparison of the proteome of HLMCs from different individuals under identical conditions will give rise to subtle, but significant variations reflecting differences in the cell donor's genotype, based either on SNPs or other polymorphisms, which may influence the biological phenotype and mast cell responses to a variety of environmental stimuli. Of particular interest in this context are donors whose cells do not respond to an IgE-mediated stimulus with mediator secretion. This phenomenon has been attributed to a defect in early IgE-mediated signal transduction and there is anecdotal evidence that the human non-releaser population is not afflicted by allergies [30]. Identification of a link between the expression of specific proteins and individual secretor status presents a self-evident target for the development of inhibitors of mast cell degranulation.

It is expected that incorporating details of protein and protein polymorphisms into protein databases may assist future drug developments. The new emerging field of pharmacogenomics, where drugs may be tailored to cohorts of people by virtue of their phenotype as assessed by 2-D gel electrophoresis, is deemed by many to be the way forward in prescribing appropriate drugs. More specifically toxicity of drugs could be minimised by screening patients, so those drugs are not prescribed to groups of patients lacking the appropriate enzymes to metabolise them. In this respect, deciphering the proteome of mast cells may assist the design of drugs targeted against the allergic response.

8. Nomenclature

2-DE	Two-dimensional electrophoresis
DE-MALDI	Delayed extraction matrix-assisted laser desorption/ionisation
HLMC	Human lung mast cell
ICAT	Isotope-coded affinity tag
PAGE	Polyacrylamide gel electrophoresis
RBL	Rat basophilic leukaemia
SAGE	Serial analysis of gene expression
SNP	Single nucleotide polymorphism

Acknowledgements

This work was supported by grants from the BBSRC and the National Asthma Campaign.

References

[1] L.H. Choe, K.H. Lee, Electrophoresis 21 (2000) 993.
[2] C. Tkaczyk, A.M. Gilfillan, Clin. Immunol. 99 (2001) 198.
[3] G. Nilsson, T. Bloom, M. Kusche-Gulberg, L. Kjellen, J.H. Butterfield, C. Sundstrom, K. Nilsson, L. Hellman, Scand. J. Immunol. 39 (1984) 489.
[4] P.N. Monk, R. Bingham, T. Ahmad, B.A. Helm, Receptor 3 (1993) 77.
[5] B.R. Bingham, P.N. Monk, B.A. Helm, J. Biol. Chem. 269 (1994) 1.
[6] A. Capron, J.P. Dessaint, M. Capron, M. Joseph, J.C. Ameisen, A.B. Tonnel, Immunol. Today 7 (1986) 15.
[7] S.N. Abraham, R. Malaviya, Infect. Immun. 65 (1997) 3501.
[8] T.E. Graham, J.R. Peiffer, R.J. Lee, D.F. Kusewitt, A.M. Martinez, T. Foutz, B.S. Wilson, J.M. Oliver, J. Immunol. 161 (1998) 6733.
[9] T. Dudler, D. Machado, R. Kolb, R. Annan, M. Gelb, M. Suter, B. Helm, J. Immunol. 155 (1995) 2605.
[10] D. Machado, D. Horton, P. Peachell, B. Helm, Eur. J. Immunol. 26 (1996) 2972.
[11] M. Gilchrist, A.J. MacDonald, I. Neverova, B. Ritchie, A.D. Befus, J. Immunol. Methods 201 (1997) 207.
[12] C. Lutzelschwab, G. Pejler, M. Aveskogh, L. Hellman, J. Exp. Med. 185 (1997) 13.
[13] K. Carroll, Ph.D. Thesis, University of Sheffield, Sheffield, 1998.
[14] H. Chen, M. Centola, S.F. Altschul, H. Metzger, J. Exp. Med. 188 (1998) 1657.

[15] B.A. Helm, Y. Ling, N. Rhodes, E.A. Padlan, in: T. Miyamato, M. Okuda (Eds.), Progress in Allergy and Clinical Immunology, Hogrefe and Huber, Seattle, Toronto, Bern, Goettingen, 1992, p. 589.
[16] K. Carroll, K. Ray, B. Helm, E. Carey, Eur. J. Cell Biol. 80 (2001) 295.
[17] D. Kouretas, J. Trnovsky, M. Holdridge, T.C. Theoharides, Sciences 49 (1991) 1289.
[18] K. Carroll, K. Ray, B. Helm, E. Carey, Electrophoresis 21 (2000) 2476.
[19] T.J. Griffin, R. Aebersold, J. Biol. Chem 276 (2001) 45497.
[20] A. Shevshenko, M. Wilm, O. Vorm, M. Mann, Anal. Chem. 68 (1996) 850.
[21] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, R. Aebersold, Nat. Biotechnol. 10 (1999) 994.
[22] V. Santoni, S. Kiefer, D. Desclaux, F. Masson, T. Rabilloud, Electrophoresis 21 (2000) 3329.
[23] P. Wickerware, P. Smaglik, Nature 433 (2001) 869.
[24] R. Malhotra, M. Ward, R.B. Sim, M.I. Bird, Biochem. J. 341 (1999) 61.
[25] O.N. Jensen, A. Podtelejnikov, M. Mann, Rapid Commun. Mass Spectrom. 10 (1996) 1371.
[26] O.N. Jensen, A. Podtelejnikov, M. Mann, Anal. Chem. 69 (1997) 4741.
[27] L.A. Huber, O. Ullrich, Y. Takai, A. Lütcke, P. Dupree, V. Olkkonen, H. Virta, M.J. DeHoop, K. Alexandrov, M. Peter, M. Zerial, K. Simons, Proc. Natl. Acad. Sci. USA 91 (1994) 7874.
[28] N.L. Anderson, Trends Anal. Chem. 1 (1982) 131.
[29] R.D. Appel, J.R. Vargas, P.M. Palagi, D. Walther, D.F. Hochstrasser, Electrophoresis 15 (1997) 2735.
[30] K.L. Nguyen, S. Gillis, D.W. MacGlashen, J. Allergy Clin. Immunol. 85 (1990) 1020.