Available online at www.sciencedirect.com

SCIENCE DIRECT

Genomics 85 (2005) 285–296

GENOMICS

www.elsevier.com/locate/ygeno

Mouse protein arrays from a T_H1 cell cDNA library for antibody screening and serum profiling

Claudia Gutjahr[a], Derek Murphy[b], Angelika Lueking[a,c], Andrea Koenig[a], Michal Janitz[a], John O'Brien[d], Bernhard Korn[e], Sabine Horn[a], Hans Lehrach[a], Dolores J. Cahill[b,c,f,*]

[a]*Max Planck Institute of Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany*
[b]*Centre for Human Proteomics, Royal College of Surgeons in Ireland, 123, St. Stephen's Green, Dublin 2, Ireland*
[c]*Ruhr-University Bochum, Medical Proteome Center, Universitätsstrasse 150, D-44780 Bochum, Germany*
[d]*Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, 123, St. Stephen's Green, Dublin 2, Ireland*
[e]*Resource Center for Genome Research, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany*
[f]*PROTAGEN AG, Emil-Figge-Strasse 76a, 44227 Dortmund, Germany*

Received 24 June 2004; accepted 10 November 2004
Available online 7 January 2005

Abstract

The mouse is the premier genetic model organism for the study of disease and development. We describe the establishment of a mouse T helper cell type 1 (T_H1) protein expression library that provides direct access to thousands of recombinant mouse proteins, in particular those associated with immune responses. The advantage of a system based on the combination of large cDNA expression libraries with microarray technology is the direct connection of the DNA sequence information from a particular clone to its recombinant, expressed protein. We have generated a mouse T_H1 expression cDNA library and used protein arrays of this library to characterize the specificity and cross-reactivity of antibodies. Additionally, we have profiled the autoantibody repertoire in serum of a mouse model for systemic lupus erythematosus on these protein arrays and validated the putative autoantigens on highly sensitive protein microarrays.

Keywords: T_H1; cDNA expression library; Automation; Mouse; High-density protein arrays; Microarrays; Protein chips; Serum profiling; SLE; Autoantibody

The mouse is the premier genetic model organism for the study of disease and development [1]. The final sequencing of the entire mouse genome [2] will greatly increase the advantages associated with mouse models. One of the next steps in expanding the usefulness of mouse models will be to make available tens of thousands of mouse proteins for large-scale high-throughput analyses, e.g., the study of antibody–protein, protein–protein, and peptide–protein interactions and enzyme activities and the characterization of antibody specificity. Such high-throughput studies are greatly simplified by protein microarray technology, whereby thousands of biomolecules are immobilized at high density onto chemically modified surfaces. The advantages of a system based on the combination of large cDNA expression libraries with microarray technology are the direct accessibility of the expressed recombinant proteins and of the DNA sequence information from a particular clone and the ability to re-array the recombinant proteins from selected clones to generate a new microarray for the creation of high-density protein arrays on glass chips [3]. For the generation of protein biochips high-throughput subcloning of open reading frames from the genome of humans, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, and *Caenorhabditis elegans* have been described [34–39]. Such recombination-based cloning approaches are strongly dependent on the progress in genome sequencing projects and the annotation of those sequences [40,37]. This means that previously uncharacterized proteins will be absent,

* Corresponding author. Fax: +353 1 402 8551.
E-mail address: chp@rcsi.ie (D.J. Cahill).

0888-7543/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2004.11.005

limiting this approach as a discovery tool. Additionally, a clear determination of the expressed sequence remains difficult due to differential splicing or posttranslational modifications. For these reasons, this approach has proved most valuable in the production of chips containing proteins from well-characterized organisms, such as *S. cerevisiae* and *C. elegans*[38,35]. This effort in creating each individual cDNA expression construct can be circumvented by the generation of arrayed cDNA expression libraries leading to thousands of cDNA expression products in parallel [4,6,41]. The use of cDNA expression libraries eliminates the need to construct individual expression clones for every protein of interest. By introducing a sequence coding for an affinity tag (e.g., His tag, GST tag) to the 5′ end of the cDNA insert, expression clones can be rapidly identified due to the detection of their His or GST fusion protein. The protein products of entire libraries can be arrayed at high density on filters and screened in parallel. The expression clones of interest can be identified and the recombinant protein purified.

An automated, robust protein expression and purification protocol to provide thousands of highly pure recombinant human proteins to generate high-density, high-content protein arrays has been developed [3,4,7]. This approach has previously been applied to generate a human fetal brain expression library, making over 10,000 different recombinant human proteins available for high-throughput studies [4]. This approach was extended to automated spotting of protein microarrays from crude lysates of the expression cultures onto membranes [7]. Furthermore, these expressed proteins are suitable for either matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or functional screening assays [5,8]. More recently, we have demonstrated that these protein arrays can be screened with peptides to find peptide-interacting proteins [9]. This human brain library has been normalized to obtain a nonredundant, unique cDNA set (Uniclone Set) using oligonucleotide fingerprinting [10]. Automated, parallel protein expression and purification were performed on 2413 protein-expressing clones, and purified proteins were immobilized on modified glass surfaces. The protein microarrays generated were shown to be suitable for determining the specificity and cross-reactivity of antibodies and to profile the antibody repertoire in serum from patients with autoimmune diseases [3]. A so-called antigen array consisting of 196 distinct biomolecules representing major autoantigens was probed with serum from patients with different autoimmune diseases like systemic lupus erythematosus (SLE), Sjögren syndrome, and rheumatoid arthritis, and distinct autoantibody patterns have been shown, indicating their potential suitability for diagnosis [42]. To study the autoantibody B cell response in acute and chronic experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, an antigen microarray consisting of 232 proteins specific for the myelin proteome was generated [43]. Putative autoantigens have been determined, followed by an antigen-specific tolerizing therapy that leads to the reduction of epitope spreading of autoreactive B cell response and a lower relapse rate.

Current alternative methods of profiling the antibody repertoire of patients with autoimmune diseases and to characterize the specificity and cross-reactivity of antibodies are very labor intensive. They involve the screening of antibodies on nonordered phage libraries or on tissue samples separated on 2D gel electrophoresis or by Western immunoblotting. Such methods have been successfully applied in the identification of potential autoantigens using autoimmune serum [11]. In contrast, combining expression libraries with high-density protein array technology would greatly simplify this process, permitting in a first step the identification of putative autoantigens from an ordered recombinant source within a relatively short time. In contrast to methods using nonordered resources, clones from such an ordered resource can be easily identified by sequencing. The subsequent use of highly sensitive protein microarrays allows systematic comparison between diseased and control human or mouse sera and the validation of previously determined putative autoantigens [12]. This approach requires only very small amounts of serum sample. However, confirmation of these putative autoantigens by additional methods such as Western immunoblot or functional assays (when available) has to be performed independent of the previously used screening methods (e.g., screening on 2D gels, high-density protein arrays).

Here, we describe the establishment of a mouse T helper cell type 1 (T_H1) protein expression library, which provides direct access to thousands of recombinant mouse proteins, in particular those associated with immune responses. These proteins can be arrayed onto surfaces, such as filters, membranes, or glass slides, and subjected to further analysis. In this work, we demonstrate the use of such protein arrays for the characterization of antibody specificity and cross-reactivity and have also shown that this protein array can be used to profile the antibody repertoire in mouse sera, including serum from a mouse model for SLE.

Results

Construction of mouse T_H1 protein expression library

To generate mouse T_H1 cells naïve, cultured mouse $CD4^+$ cells were stimulated with antigenic peptide OVA323-339 and antigen-presenting cells in the presence of interleukin-12 (IL-12). In addition, endogenous IL-4 was neutralized by addition of a monoclonal anti-IL-4 antibody [13]. T_H1 induction was confirmed by controlling the cell population for the absence of IL-4-producing cells, and the expression of interferon-γ (IFN-γ) in 99% of cells. The mRNA was isolated from these mouse T_H1 cells and reverse transcribed into cDNA. This cDNA was directionally cloned into the *Escherichia coli* expression vector pQE-30NAS-

TattB (GenBank Accession No.AY386205), a modified form of the previously described pQE-30NST vector [4]. This vector enables IPTG-inducible expression of N-terminal RGSHis$_6$ fusion proteins and is compatible with the Gateway system (Invitrogen).

This T_H1 library, called m$T_H1$long821, was then transformed into electrocompetent *E. coli* SCS1 cells, already carrying the plasmid pSE111, which contains the *lacIq* repressor and the *argU* gene for rare tRNAs coding for arginine [14], thus improving expression of mammalian genes in this bacterial system.

Initially, 100 clones from this library were picked and the average insert size of the library was determined to be 1.0 kb, with the insert size ranging from 0.5–5.0 kb, as cDNA products under 0.5 kb were size selected on Sephacryl S500-HR columns and removed from the library (data not shown). As expected, a high number of the clones (21%) code for proteins associated with T_H1 cells. For example, the most common known sequence identified is for the cytokine IFN-γ, which is actively secreted by T_H1 cells. Of particular interest for the study of the proteome of any particular system is the presence of clones coding for previously uncharacterized proteins. We found that 13 of the 100 clones sequenced are associated with proteins that have no described function in GenBank, where they are either unknown or have not yet been functionally characterized. These results indicate that the cDNA library can provide us with a large repertoire of mouse proteins, in particular those associated with T cell activity, including previously uncharacterized proteins. The quality of the expression library having been assured, a total of 65,100 clones were robotically picked from the library and transferred into 182 × 384-well microtiter plates.

Generation and analysis of T_H1 expression subset

The next step in characterizing the T_H1 protein expression library was the identification of the subset of clones with inserts in the correct reading frame for heterologous expression of mouse proteins. We have previously demonstrated that it is possible to identify cDNA clones with inserts that are in the correct reading frame with respect to their N-terminal RGSHis$_6$ tag [4]. To achieve this, we generated protein arrays on PVDF membranes, as previously described, and screened with an antibody specific for the RGSHis$_6$ epitope, which specifically recognizes the N-terminal RGSHis$_6$ tag of the expressed recombinant proteins. Inserts in the incorrect reading frame will, due to the presence of stop codons, lead to short and unstable protein products, which are degraded in *E. coli*[15]. To achieve this selection, from the entire library, 65,100 T_H1 cDNA clones were gridded onto PVDF filters generating high-density arrays using a robotic system [16]. Following overnight growth of the colonies, protein expression was induced by the addition of IPTG, as previously described [4]. Then, the filters were incubated with the anti-RGSHis$_6$ antibody and detected with an alkaline phosphatase-labeled secondary antibody. Positive clones were identified on the high-resolution images (Fig. 1) using VisualGrid software (GPC-Biotech). A total of 11,250 clones were detected as expressing RGSHis$_6$-tagged proteins, which represents 17% of the 65,100 clones analyzed.

The 11,250 clones representing the protein expression subset of the original library were picked and re-arrayed into 384-well plates. PCR analysis of 100 clones of this expression subset revealed an average insert size of 1.0 kb, ranging from 0.5 to 5 kb, which agrees with the data from

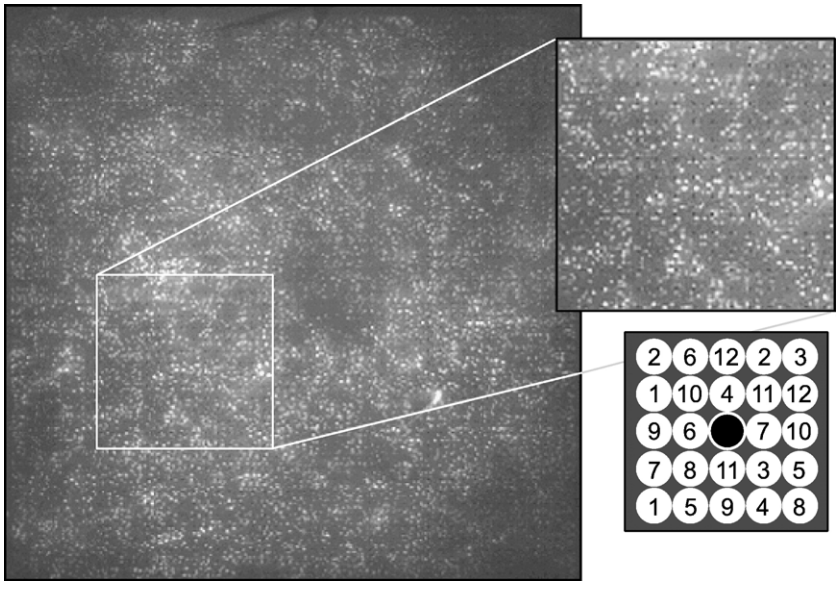


Fig. 1. High-density array of mouse T_H1 cDNA clones. 65,000 clones of the cDNA library were gridded onto three PVDF membranes in a 5 × 5 pattern (right). One filter contained 27,648 clones (left). Clones expressing recombinant RGSHis$_6$-tagged mouse proteins were identified using an anti-RGS-His$_6$ antibody, followed by detection using an alkaline phosphatase-labeled secondary antibody. Positive signals on the filters were visualized with a high-resolution CCD imaging system, during exposure of the filters to long-wave UV light. Clone identification was carried out using VisualGrid software.

the initial total cDNA. Four hundred twelve clones were sequenced and BLASTed against GenBank. In Table 1, the most common proteins are listed. Analysis of 193 sequences confirmed that 56% of the cDNA inserts coding for proteins are in the correct reading frame with the $RGSHis_6$ tag. In addition, all sequenced data were used to compile a functional ontology of the library (Fig. 2). Fifty-three percent of the clones code for proteins that are involved in cell growth and maintenance, 18% are involved in cell communication, 4% in developmental processes, and 0.5% in cell death. Twenty-three percent of the clones code for proteins with no described function.

To confirm the expression of recombinant mouse proteins in this subset of 11,250 clones, 96 clones were expressed and the $RGSHis_6$-tagged proteins were purified, in parallel, under denaturing conditions by Ni–NTA-immobilized metal-affinity chromatography [17]. The purified proteins were separated by SDS–PAGE and recombinant mouse proteins were observed in 68% of these clones. The average size of the recombinant proteins is approximately 35 kDa, with protein sizes ranging from 10 to 80 kDa. Western immunoblot analysis was performed on a selection of 48 purified proteins using an antibody against the $RGSHis_6$ epitope and confirmed the specific expression and purification of $RGSHis_6$-tagged fusion proteins (Fig. 3).

Table 1
Functional classification of clones from T_H1 library

Protein	Accession No.	No. clones
T cell receptor β chain V region C5 precursor	P04213	6
Tropomyosin α4 chain	P07226	5
Small inducible cytokine A4 precursor	P14097	5
Interferon-γ precursor	P01580	4
Transcription factor jun-B	P09450	4
Dual specificity protein phosphatase 2	Q05922	4
T cell receptor β chain T17T-22 precursor	P11364	3
Glyceraldehyde-3-phosphate dehydrogenase	P16858	3
Lysyl-tRNA synthetase	Q15046	3
Probable RNA-dependent helicase p68 (DEAD-box protein p68)	Q61656	3
Cyclin pch1	O74627	2
Osteopontin precursor	P10923	2
Secretory granule proteoglycan core protein precursor	P13609	2
High-mobility-group protein HMG-Y	P17095	2
Peptidylprolyl *cis-trans* isomerase A	P17742	2
F-actin capping protein α2 subunit	P47754	2
NF-κB inhibitor β	Q60778	2
Tyrosine-protein kinase JAK3	Q62137	2
E1B protein	P03245	1
Orphan nuclear receptor HMR	P12813	1

The 5′ sequences of 412 clones were determined and the results were used for BLAST searches against the public databases including GenBank [20]. The most common proteins found in the 412 clones analyzed are shown.

Screening of antibodies specific for proteins of inflammatory and immune processes

To screen thousands of proteins in parallel, the clones of the protein-expressing subset described above were gridded onto PVDF membranes to generate high-density protein arrays.

To demonstrate the potential of such T_H1 protein arrays to characterize the specificity and cross-reactivity of antibodies, we examined three known T cell antibodies against the following proteins: IFN-γ, interleukin 12 receptor (IL-12R), and macrophage inflammatory protein 1β (MIP-1β; Fig. 4A). The protein arrays were screened separately with each of these monoclonal antibodies. Since the secondary antibodies that were used to detect the monoclonal antibodies might give some nonspecific background signal, control incubations with the secondary antibody alone were performed. Clones that were detected in the control as well as the anti-IFN-γ, anti-IL-12R, and anti-MIP-1β incubations were discarded. All clones expressing a protein found to bind specifically to one of the tested monoclonal antibodies were sequenced. The monoclonal anti-MIP-1β antibody recognized its antigen, which was present 22 times in the protein-expressing subset of this redundant mouse T_H1 cDNA library. Western blot analysis shows that this antibody recognized only 1 additional protein, a phosphoserine aminotransferase, indicating a high specificity of this antibody (Fig. 4B). In contrast, the polyclonal anti-IL-12R antibody binds to 18 proteins but none of these proteins represents the corresponding antigen (data not shown), indicating a low specificity of this antibody. Western blot analysis confirmed that the anti-IFN-γ antibody recognized two clones, which corresponded to its antigen, IFN-γ. This antibody also shows weak cross-reactivity to 3 other proteins, namely glutathione peroxidase 4, galactosidase-β1 and mouse Ig rearranged κ-chain mRNA V region (Fig. 4C). No homology between these proteins and IFN-γ was found.

These results demonstrate that the commercially available antibodies cross-reacted to a number of proteins in the array, which was confirmed by Western immunoblotting and demonstrates an application of this mouse protein array. We also saw that the monoclonal antibodies were more specific than the polyclonal antibodies and, in general, we have seen that the specificity of polyclonal antibodies is dependent on its method of purification.

Profiling the autoantibody repertoire of a mouse model for systemic lupus erythematosus

Screening protein arrays with sera from large numbers of autoimmune patients has been successfully performed

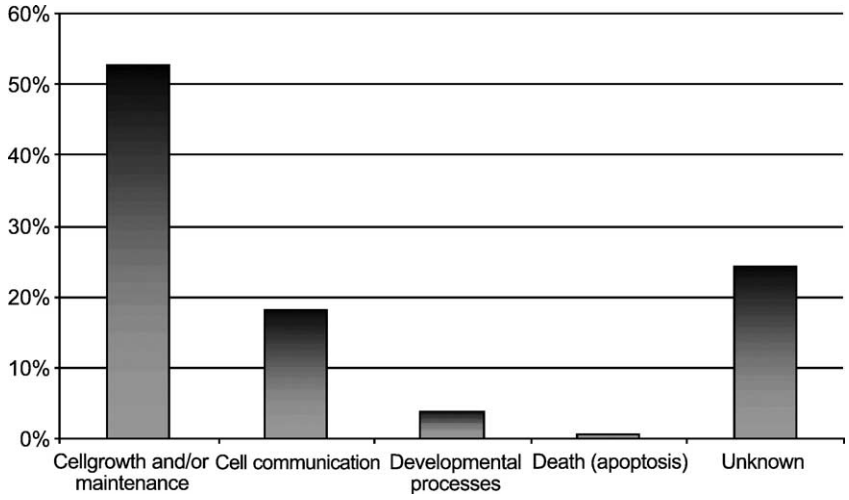
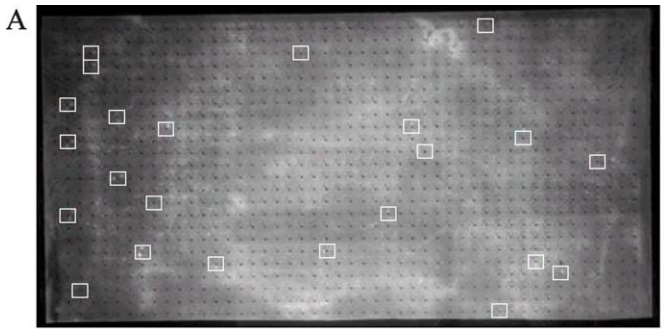


Fig. 2. Functional classification of clones from T_H1 library. The graph shows the results of analysis of 412 clones from the expression subset of the library using an ontology database (www.protogo.cs.huji.ac.il).

and recently described [3,18] and the results suggest the suitability of protein arrays for the identification of potentially new autoantigens, which may have applications in assisting in the diagnosis and subtyping of autoimmune diseases based on the presence of specific autoantibodies. Here, we use the mouse T_H1 expression library and combined it with screening sera from mouse models specific for related human autoimmune diseases to test if this approach would have potential to add impact to understanding the disease mechanisms and, perhaps, current diagnosis and subtyping of human autoimmune diseases, based on the presence of specific autoantibodies [18]. In this proof-of-principle experiment, we demonstrate the use of protein arrays of the T_H1 expression library for the analysis of autoantibodies in serum samples of a mouse model for SLE. The hybrid mice from strain NZB × NZW (New Zealand Black × New Zealand White) are one of the best animal models for SLE [19]. Pooled sera from 13 female mice, each 3 to 4 months of age, of the strain NZB × NZW were incubated onto a protein array of the T_H1 expression library. The data sets obtained from SLE serum incubations were compared to those from control sera from female NZW × Balb/c mice of the same age, which represent the healthy control, and the data sets were also compared to background incubations with the secondary antibody. From these results, 31 proteins detected by the sera from the SLE mouse model were identified.

The 5′ sequences of these 31 clones were determined and the results were used for BLAST searches against the public databases, including GenBank [20], which are shown in Table 2. Eight of these clones express the proteasome α7/C8 subunit protein. Seven clones carry the sequence coding for ATP synthase, a H^+ transporting mitochondrial F1 complex, but were not in the correct reading frame with respect to the $RGSHis_6$ tag. A further 3 clones code for a tyrosine phosphatase protein. The following proteins were also each identified once: lectin, galactose-binding, soluble 3 (Lgals3); zinc finger protein 216 (Zfp216); and CD27-binding protein. Other single clones identified, with weak signals, include a human intramembrane protease (IMP2) and endogenous ecotropic murine leukemia proviral locus 3 (Emv-3) envelope glycoprotein. Three clones coding for ribosomal proteins (Rps16 and 2 for Rps27a) were identified and 2 other clones coding for ubiquitin A 52-residue ribosomal protein fusion product 1 were recognized, and 3 clones represent proteins with no known function, with 1 of them not in the correct reading frame. These clones also give us an indication of the diversity of proteins in this T_H1 cDNA library.

Fig. 3. Expression and purification of recombinant proteins from the mouse T_H1 library. Recombinant His-tagged proteins were expressed and purified from 48 expression clones. Proteins were separated by SDS–PAGE, immunoblotted, and detected with immunochemical means using an anti-$RGSHis_6$ antibody. The proteins range in size from 10 to 80 kDa. His-tagged recombinant proteins were detected in all clones and correspond to the sizes observed when proteins were Coomassie stained.



Recombinant Proteins recognised by anti-MIP1-β	No. Clones	Accession No.
Mouse macrophage inflammatory protein 1-beta	22	M35590
Mus musculus phosphoserine aminotransferase	1	BC004827

Recombinant Proteins bound by anti-IFN-γ	No. Clones	Accession No.
Mus musculus interfer on gamma	2	XM_125899
Mus musculus, galactosidase, beta 1	1	BC028875
Mus musculus, glutathione peroxidase 4	1	NM_008162
Mouse Ig rearranged kappa-chain mRNA V-region	1	M63550

Fig. 4. High-density protein filters containing 11,250 mouse protein expression clones from the T_H1 library were screened with monoclonal antibodies. (A) A filter screened with anti-MIP-1β antibody. (B) Proteins identified on the filter were expressed and purified and the results were confirmed in Western immunoblots. Each clone was sequenced. Anti-MIP-1β binds 22 clones encoding MIP-1β and 1 clone encoding phosphoserine aminotransferase. (C) In the case of the anti-interferon-γ antibody, 5 clones were identified, 2 coding for interferon-γ and 3 clones coding for other proteins.

To confirm the binding of the antibodies in the mouse sera to these recombinant proteins, these proteins were expressed from bacterial clones and purified, in parallel, under denaturing conditions corresponding to the state of the proteins immobilized on the PVDF protein arrays. Microscope glass slides coated with a three-dimensional nitrocellulose-based matrix were prepared. Then each purified recombinant protein sample was spotted, in duplicate, and at three concentrations, undiluted and 1:2 and 1:5 dilution corresponding to approximately 30, 15, and 6 fmol/spot concentration, respectively, to generate highly sensitive, purified mouse protein microarrays (Fig. 5A). These mouse protein microarrays were then incubated with the same pools of SLE mouse model and control sera, at the same serum dilution for the same time as previously used in the initial screen performed on the mouse PVDF protein arrays (Fig. 5B).

The comparison of signal intensities of spots from SLE pooled and control pooled sera on the mouse array generated the following results: the ubiquitin A 52-residue ribosomal protein fusion product 1, Lgals3, and Rps27a gave a positive result in all three dilutions. Four additional proteins, namely Zfp216, Emv-3 envelope glycoprotein, the protein that shows weak homology to a human intramembrane protease (IMP2), and the CD27-binding protein, were positive at two dilutions, those of the highest and middle protein concentrations. Rps16, the proteasome α7/C8 subunit, and one protein with no known function were positive at one protein concentration, the highest.

Where multiple clones that express the same mouse recombinant protein were identified, only one was included in the further analysis using Western immunoblot. For this Western immunoblot analysis, equal concentrations of the 14 different, purified proteins (Fig. 6A) were used, followed by subsequent incubation with the pooled mouse SLE (Fig. 6B) and mouse control sera (Fig. 6C). Serum antibodies binding to proteasome α7/C8 subunit, to Rps27a, and to ubiquitin A 52-residue were confirmed. No binding of antibodies in the control serum to these proteins was observed. Lectin- and galactose-binding protein was weakly detected by antibodies in the control mouse sera, but much stronger with the SLE mouse sera, suggesting a disease-specific role. In contrast, similar binding of control and SLE sera to the clones carrying the sequence coding for the ATP synthase protein, the Rps16, the protein tyrosine phosphatase, and ES cell cDNA (RIKEN full-length enriched library, DNA sequence from clone RP-23-42815) was observed, indicating no disease-specific relevance of these proteins.

This analysis confirmed the following proteins as potential targets for autoantibodies in SLE mice: lectin, galactose-binding protein, soluble 3; ubiquitin A 52-residue ribosomal protein fusion product 1; and ribosomal protein RPS27a.

Discussion

One of the major challenges to understanding better the function and biology of any particular animal, organ, or cell

Table 2
Sequence analysis of 31 clones from the TH1 expression subset

Name	No. of clones	Accession No.	Microarrays Average$_{protein}$ SLE pool/average$_{protein}$ control pool			Western immunoblot SLE pool/ control pool
			1:1	1:2	1:5	
Proteasome α7/C8 subunit	8	AF055983	7.3	1.3	1	Yes/no
Protein tyrosine phosphatase, receptor type, C polypeptide-associated protein	3	BC013273	1.8	1.1	0.6	Yes/yes
ATP synthase, H^+ transporting mitochondrial F1 complex, β subunit	7	BC037127	0.25	1.3	0.8	Yes/yes
Lectin, galactose binding, soluble 3 (Lgals3)	1	XM_127681	3.6	8.8	10	Yes/yes
Ribosomal protein S16 (Rps16)	1	XM_289707	3.2	0.5	1	Yes/yes
Ribosomal protein S27a (Rps27a)	2	NM_024277	14.3	22.7	26.5	Yes/no
Ubiquitin A 52-residue ribosomal protein fusion product 1	2	BC054413	7.6	24.3	8	Yes/no
Cd27 binding protein (Hindu God of destruction), Siva-pending	1	BC037127	16	4.3	1.6	Yes/no
Endogenous ecotropic murine leukemia proviral locus 3 (Emv-3) envelope glycoprotein; glycoprotein (gp70)	1	L37057	2.12	19.4	0.2	No/no
Zinc finger protein 216 (Zfp216)	1	NM_009551	6.6	8	0.8	No/no
Human intramembrane protease (IMP2)	1	AY169313	3.4	2	0	Yes/no
RIKEN cDNA 3110006P09.	1	BC037127	5.6	0.5	1	No/no
ES cells cDNA, RIKEN full-length enriched library, clone 2410080P20; FLJ21702 FIS, clone COL09874	1	AK075958	0.7	1.3	1.2	Yes/yes
DNA sequence from clone RP23-428I5 on chromosome 11	1	AL645847	1	0.7	2.9	Yes/no

The clones were detected by the analysis of autoantibodies in serum samples of a mouse model for systemic lupus erythematosis (SLE). 31 proteins detected by the SLE sera were identified. The 5′ sequences of these 31 clones were determined and the results were used for BLAST searches against the public databases including GenBank [20].

system is the ability to link DNA sequence information directly with protein function for thousands of proteins to facilitate high-throughput characterization of thousands of proteins [12,21].

Using a similar approach, as previously described by Büssow and co-workers, we have established a protein expression library from murine T_H1 cells that provides a source of approximately 11,000 redundant recombinant mouse proteins, representing the first catalogue of thousands of mouse proteins. By cloning T_H1 cDNA into the pQE30NAST vector, expression of the RGSHis$_6$ fusion proteins was easily detected using an anti-RGSHis$_6$ antibody. Combining conventional cDNA library techniques with automation, 65,000 clones of the T_H1 cDNA library clones were picked in the microtiter plates. Protein arrays were generated and were subsequently screened for protein expression using an antibody specific for the RGSHis$_6$ tag, and 11,013 protein-expressing clones were identified. These clones were robotically picked and re-arrayed into a protein expression subset. Based on DNA sequence and protein expression analysis, this expression subset contains approximately 56% protein-expressing clones, with an average protein size of 35 kDa, comprising 35% full-length clones.

The analysis of approximately 412 clones (see Table 1) shows proteins involved in housekeeping, ribosomal, and cell cycle functions and includes additionally 23% proteins associated with the immune response, suggesting this mouse T_H1 library is a valuable source of thousands of mouse proteins for studies focusing on the immune system. In addition, the library contains a large percentage (23%) of functionally uncharacterized or unknown proteins. Therefore, this library represents a unique resource for the study of mouse proteins in general. Such a source of proteins will enable high-throughput characterization of proteins, including the study of protein–protein and peptide–protein interactions, determination of antibody specificity, and profiling the antibody repertoire in serum of mouse models.

We have demonstrated the characterization of antibody specificity and cross-reactivity on high-density protein arrays of the mouse T_H1 expression library. Antibodies are commonly used as research tools and in diagnostic assays in many different formats [22], requiring a high assay sensitivity and specificity. Also since antibodies are used for the generation of antibody arrays, which consist of immobilized antibodies that can be used to profile the expression of proteins, for example, in body fluids or tissue

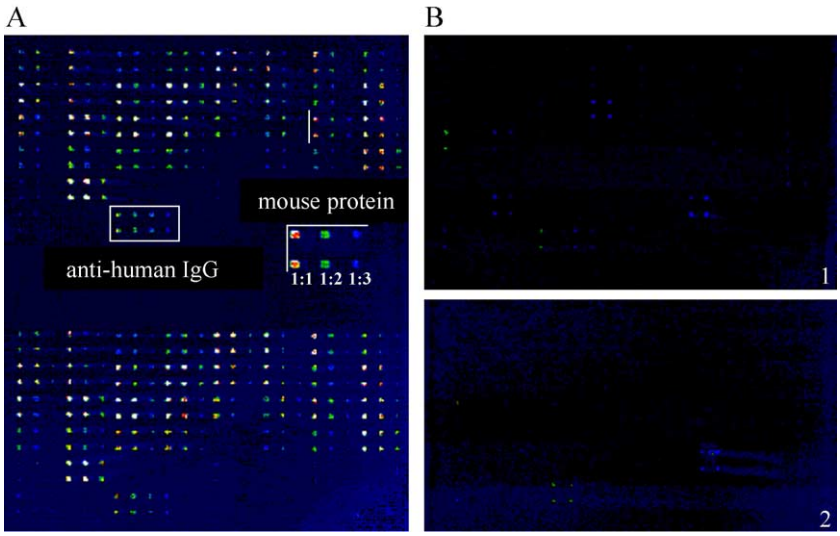


Fig. 5. Protein microarray with 31 proteins of the T_H1 expression library. The proteins were detected by analysis of autoantibodies in serum samples of a mouse model for SLE. The proteins were spotted in three dilutions and in duplicate. (A) Microarray incubated with an antibody against the $RGSHis_6$-tag of recombinant proteins. (B) The same field of two different microarrays, which were incubated with pooled sera. Field 1 was incubated with sera of the mouse model for SLE (NZB × NZW) and field 2 with the serum pool of the mouse control strain (NZW × Balb/c).

extracts, it is of increasing importance to analyze the antibodies for their specificity against a large number of different proteins.

We examined two monoclonal antibodies against mouse IFN-γ and MIP-1β and one polyclonal antibody against mouse IL-12R. All these antibodies were commercially available and designed for use in Western immunoblotting, indicating their ability to recognize specifically denatured, linear epitopes in their cognate protein. It has been shown that the monoclonal antibody against MIP-1β was highly specific for the MIP-1β protein, whereas the monoclonal antibody against IFN-γ recognized additional other proteins, indicating a certain cross-reactivity. In contrast, the affinity-purified polyclonal antibody shows a high cross-reactivity. These results demonstrate that monoclonal antibodies may give higher specificity and lower cross-

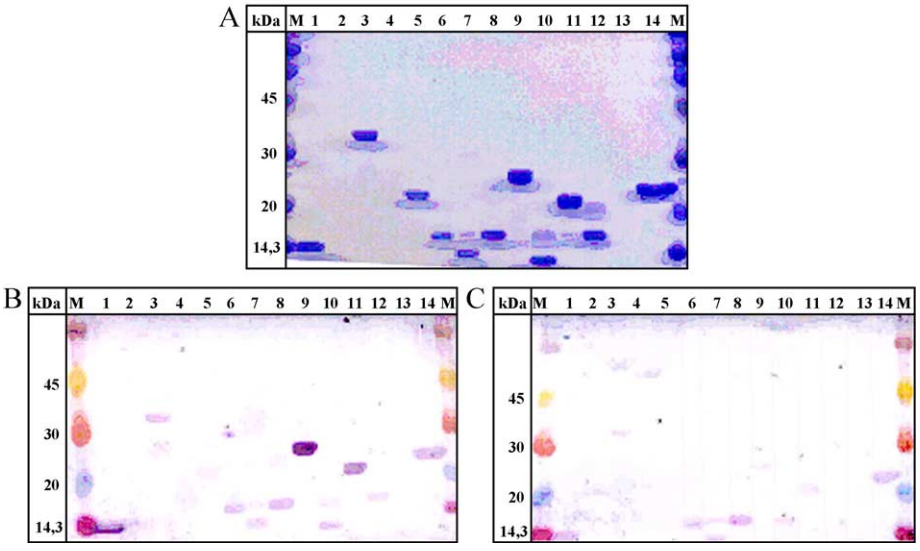


Fig. 6. (A) SDS–PAGE and (B and C) Western immunoblot with 14 proteins of the T_H1 library. The proteins were detected by serum screening on microarrays. The Western immunoblots were incubated with pools of murine sera of a mouse model for SLE (B) and of the control strain (C). The proteins were distributed as followed: Lanes 1, ATP-synthase, H^+ transporting mitochondrial F1 complex; lanes 2, mouse endogenous ecotropic murine leukemia proviral locus 3 (Emv-3) envelope glycoprotein; lanes 3, proteasome α7/C8 subunit; lanes 4, zinc finger protein 216 (Zfp216); lanes 5, RIKEN cDNA 3110006P09; lanes 6, protein tyrosine phosphatase receptor type C; lanes 7, ES cell cDNA, RIKEN full-length enriched library; lanes 8, ribosomal protein S16; lanes 9, ribosomal protein S27; lanes 10, DNA sequence from clone RP23-42815; lanes 11, ubiquitin A 54-residue ribosomal protein fusion product; lanes 12, CD27-binding protein; lanes 13, human intramembrane protease (IMP2); lanes 14, lectin, galactose binding, soluble 3 (Lgals3).

reactivity than polyclonal antibodies and that the specificity of polyclonals is dependent on the quality of the purification methods used. For antibodies used as research tools in diagnostic assays or for immobilization onto antibody microarrays, best results will be obtained using antibodies that are shown to be specific for their target protein when screened against large numbers of proteins, as described here.

Screening protein arrays with sera from autoimmune patients, for example with rheumatoid arthritis and alopecia, has shown to be useful in identifying potential disease-associated autoantigens [3]. Applying this technology to screening sera from mouse models, such as models of autoimmune diseases, against the mouse T_H1 expression library may add to the understanding of the mechanisms involved in such diseases or potentially may add to current diagnosis and subtyping of human autoimmune diseases, as various mouse models exist for a number of autoimmune disorders.

Here, we analyzed a model for the human autoimmune disorder SLE, which is a polymorphic autoimmune disease caused by unknown factors. It is characterized by the presence of different autoantibodies against nuclear and cytoplasmic antigens, such as ribonucleoprotein Sm, La-SSB, and anti-histone, as well as autoantibodies against dsDNA [23]. We profiled the autoantibody repertoire of the SLE mouse model and compared this with the healthy control mouse and identified four different putative autoantigens.

Here, we identified mouse lectin- and galactose-binding protein soluble 3 protein when mouse PVDF protein arrays were screened with sera from the mouse model for SLE. These results were confirmed on mouse protein microarrays and by Western immunoblotting analysis. Autoantibodies against Lgals3 have been described in human SLE and other autoimmune diseases [24]. Analysis of the protein sequences of the human and the mouse proteins show an identity of 66%. We have also detected a gene coding for the proteasome α7/C8 subunit protein on the protein filters and have confirmed its specificity on protein microarrays and by Western immunoblotting analysis. In humans, proteins of the 20S proteasome, such as the subunit C9α, have been frequently recognized in different autoimmune diseases, including SLE [25,26], suggesting these proteins may have potential as marker proteins for general autoimmune inflammation and cell damage [27]. In this context, we do not know if the proteasome α7/C8 subunit is specific for SLE or is detected in a more general way, in other mouse models of autoimmune diseases.

Additionally, autoantibodies against ribosomal proteins of the 60S subunit and against P proteins have been reported [28–30]. Here, we have identified mouse autoantibodies against Rps27a and ubiquitin A 52-residue ribosomal protein fusion product 1, which belong to the 40S subunit. Using the SLE mouse model, the results obtained seem to be comparable to the results from studies with human patients. However, both Rps27a and ubiquitin A 52-residue ribosomal protein fusion product 1 identified here have not been previously reported in a human system. These proteins may be potential new autoantigens or mouse proteins related to the human immune system.

However, it is unclear whether the identified potential autoantigens are functionally related to the autoimmune disease. Comparing various putative autoantigens specific for other autoimmune diseases, such as rheumatoid arthritis, only some autoantigens are described with known functional significance in the disease. For others no functional significance was found for these autoantigens [44]. Interestingly, however, some of those autoantigens with unknown function in relation to the autoimmune disease have still successfully been used as biomarkers of the disease [45].

Materials and methods

Isolation of $CD4^+$ cells

Mice homozygously transgenic for the DO11.10 αβ-T cell receptor (OVA-TCR^{tg}) [31] on Balb/c background were bred under specific-pathogen-free conditions in laminar flow incubators. Mice (8–12 weeks of age) were sacrificed by cervical dislocation and, subsequently, their spleen cells (SC) were isolated. OVA-TCR^{tg} SC were stained with FITC-conjugated anti-CD4 mAb and MultiSort anti-FITC microbeads (Miltenyi Biotec). $CD4^+$ cells were isolated by positive selection on VS^+ columns using the high gradient magnetic cell separation system (Miltenyi Biotec) to a purity of 99%.

Polarization of naïve T_H cells into T_H1 cells

Naïve $CD4^+$ cells were cultured at 1 to 2 × 10^6 cells/ml in complete RPMI 1640 medium containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.3 mg/ml glutamine, 10 μM 2-mercaptoethanol, and 5% FCS (v/v). The cells were stimulated for 1 week with antigenic peptide $OVA_{323–339}$ (Neosystem) at a concentration of 1 μM and antigen-presenting cells (T-cell-depleted spleen cells) in the presence of recombinant murine IL-12 (R&D Systems) at a concentration of 100 U/ml. A neutralizing anti-IL-4 mAb, 11B11 [13], at 10 μg/ml was also added to neutralize any endogenous IL-4. Following 1 week of culture, no IL-4-producing cells (<0.1%) were detectable by intracellular staining [32] using mAb 11B11. In contrast, most of the cells (99%) expressed IFN-γ as detected by intracellular staining using AN18.17.24 mAb [33], thus indicating the T_H1 phenotype of the cells. Intracellular staining was estimated by cytometry using FACSCalibur and CELL-Quest research software (Becton–Dickinson). Following 1 week in culture, the polarized T_H cells were harvested and

subjected to cytoplasmic RNA isolation. In brief, 3×10^7 pelleted cells were resuspended in extraction buffer (140 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.6), 0.5% NP-40) and incubated on ice for 5 min. Nuclei and cell debris were separated by 25,000*g* for 5 min and the remaining proteins in the cytoplasmic fraction were inactivated by addition of SDS (0.57% (w/v) final conc) and proteinase K (0.57 μg/μl final conc) and incubation at 56°C for 30 min. After two phenol/chloroform extractions, the RNA was precipitated using ethanol and NaAc (pH 5.2) and washed with 80% (v/v) ethanol. The RNA was resuspended in 50 μl H_2O.

Poly(A)$^+$ RNA was selected using oligo(dT) beads from Dynal (Hamburg, Germany) according to the manufacturers' recommendations. One microgram of poly(A)$^+$ RNA was converted to cDNA by 400 U Superscript reverse transcriptase (Invitrogen, Carlsbad, CA, USA), using the buffer provided by the supplier and 1 μg GCGGCCGC$(T)_{21}$V primer. Second-strand synthesis was done according to [46]. *Sal*I adaptors were ligated and the cDNA was cleaved by *Not*I. Following size selection on Sephacryl S500-HR columns, cDNA pools greater than 500 bp were directionally ligated to a *Sal*I/*Not*I-prepared pQE30NASTattB vector, a modified form of the pQE30NST vector (www.rzpd.de/info/vectors/pQE30NST.shtml).

Strains, media, and transformation

E. coli SCS1 cells (Stratagene) carrying the plasmid pSE111 with the *lacI*^{Q} repressor and the *argU* gene for rare arginine tRNA [14] were transformed by electroporation (GenePulser; Bio-Rad). Transformants were selected on 2YT medium (1.6% (w/v) Bacto tryptone, 1% (w/v) Bacto yeast extract, 0.5% (w/v) NaCl, 1.5% (w/v) agar) supplemented with 100 μg/ml ampicillin, 15 μg/ml kanamycin, and 2% (w/v) filter-sterilized glucose. The clones were picked into 384-well microtiter plates (Genetix) containing 2YT medium and, additionally, containing 100 μg/ml ampicillin, 15 μg/ml kanamycin, 2% (w/v) filter-sterilized glucose and freezing mix (0.4 mM $MgSO_4$, 1.5 mM Na_3-citrate, 6.8 mM $(NH_4)_2SO_4$, 3.6% (v/v) glycerol, 13 mM KH_2PO_4, 27 mM K_2HPO_4, pH 7.0).

The mouse strain used was New Zealand Black × New Zealand White).

To measure the disease activity the concentration of the autoantibodies was clearly defined (especially anti-dsDNA, anti-smD). Additionally there were defined clinical parameters, for example urea and creatinine, to test the function of the kidney.

Generation of the T_H1-expression cDNA library

The oligo(dT)-primed cDNA library was directionally (*Sal*I/*Not*I) cloned into the expression vector pQE30NASTattB (a modified pQE30 from Qiagen) for IPTG-inducible expression of His_6-tagged fusion proteins. Following transformation into *E. coli* (strain SCS1), clones were plated onto agar plates (230 × 230 mm; Genetics) and grown overnight at 37°C. Using a picking robot, about 65,000 clones were picked in 384-well microtiter plates and incubated overnight. For sequencing, the cDNA inserts were amplified directly from the clone by PCR using primers pQE65 (TGAGCGGATAACAATTTCACACAG) and pQE276 (GGCAACCGAGCGTTCTGAAC).

The T_H1-expression subset is managed by the Resource of the German Human Genome Project (http://www.rzpd.de).

Generation of high-density protein arrays

High-density protein arrays on a PVDF membrane containing 27,648 clones, in duplicate, per array, were generated as previously described [4].

Western immunoblot analysis

The proteins were purified under denaturing conditions, separated by SDS–PAGE, and transferred to PVDF membranes for Western immunoblot analysis. The membranes were incubated with antibodies and sera under the same conditions as the incubation on the PVDF protein arrays and protein microarrays on coated glass slides.

Antibody and serum screening

Prior to antibody screening, the filters were treated as previously described [4]. Following blocking with 3% (w/v) milk powder in TBST (TBS + 0.1% Tween 20) for 1 h at room temperature, the filters were incubated overnight with the primary antibody, i.e., anti-IFN-γ monoclonal antibody, 1:1000 dilution in blocking buffer (Clone H22.1, sc-12743; Santa Cruz Biotechnology, Inc); anti-MIP-1β monoclonal antibody (Clone A65-2; BD Pharmingen), 1:1000 dilution in blocking buffer; anti-IL-12R polyclonal antibody (Clone CC-20; Santa Cruz Biotechnology, Inc.), 1:1000 dilution in blocking buffer; anti-RGSHis_6 (Qiagen), 1:2000 dilution; or pools of sera, diluted 1:100 in blocking solution. This incubation with primary antibody was followed by three washes, for 20 min each, in TBST, and the protein arrays were then incubated with the appropriate secondary antibody (alkaline phosphatase (AP)-conjugated anti-rat IgG from Sigma, anti-rabbit IgG–AP from Boehringer Mannheim GmbH, anti-Armenian hamster–AP from Jackson ImmunoResearch Laboratories) for 1.5 h. Following three washes for 20 min in TBST, the filters were incubated for 10 min in AP buffer (1 mM $MgCl_2$, 100 mM Tris–Cl, pH 9.5) and 5 min in 0.125 mM Attophos in AP buffer. Filters were illuminated with long-wave UV light and images were taken using a high-resolution CCD camera (Fuji). Image analysis was done using VisualGrid.

Generation of protein microarrays

The FAST slides (Schleicher & Schuell) were placed in a Q-Array system (Genetix, New Milton, UK), equipped with humidity control (56%) and 16 blunt-ended stainless steel print tips with a tip diameter of 150 μm. All proteins were spotted at three different concentrations (undiluted (30 fmol/spot) and 1:2 (15 fmol/spot) and 1:5 (6 fmol/spot) in blocking buffer). Each protein microarray includes several control spots, e.g., human or mouse IgG in four concentrations (1:500, 1:1000, 1:2000, and 1:5000). For the protein chip, a 6 × 6-spot pattern was printed onto the slides including all protein dilutions, spotted in duplicate. In all cases, each spot was loaded once, by transferring 2 nl per spot with 150-μm solid pins (Q-Array; Genetix).

Serum screening on protein microarrays

After spotting, the protein chips were blocked in 3% (w/v) milk powder/TBST at room temperature for 2 h and, subsequently, the pooled mouse sera were added (diluted 1:100 in 3% (w/v) milk powder/TBST). The protein chips were incubated at 4°C in a humidified atmosphere for 16 h. Then, following three 30-min TBST washes, the protein chips were incubated with the secondary antibody (Cy5-labeled rabbit anti-mouse IgG from Dianova) at 1:800 dilution in 3% (w/v) milk powder/TBST. The arrays were washed three times, each in TBST for 20 min. All antibody incubation steps were performed in the dark for 1 h and were carried out in a 200 μl volume on the protein chips, which were covered by a coverslip. The results on the protein chips were detected using a confocal microarray reader (ScanArray 4000; Perkin–Elmer Life Science).

Bioinformatical analysis

Image analysis was performed using GenePix Pro 3.0 (Axon) or ScanArray Express (Perkin–Elmer Life Science) and the median intensity (background subtracted) was determined for each protein feature on the chip. The average value of the protein duplicates was calculated, followed by determination of the quality of the screen, by calculating the coefficient of variation of the control protein human IgG. These values were also used for interchip normalization.

The factor ($average_{protein}$ × SLE pool/$average_{protein}$ × control pool) was calculated. The proteins with values of at least 2 were taken as positive.

Web site references

www.rzpd.de/info/vectors/pQE30NST.shtml.
www.protogo.cs.huji.ac.il.

Acknowledgments

We thank Tiina Humaljoki (DRFZ Berlin) for providing the serum samples and Thorsten Stamm (DRFZ Berlin) for providing induced T cells. This work was funded by the Bundesminesterium für Bildung und Forschung and the Deutsches Humanes Genomprojekt DHGP (BMBF Grant 01KW9913) and the Max-Planck-Gesellschaft. D.J.C. gratefully acknowledges funding from the Health Education Authority and Science Foundation Ireland, Dublin, Ireland.

References

[1] A.S. Perkins, Functional genomics in the mouse, Funct. Integr. Genom. 2 (2002) 81–91.
[2] R.H. Waterston, et al., Initial sequencing and comparative analysis of the mouse genome, Nature 420 (2002) 520–562.
[3] A. Lueking, et al., A nonredundant human protein chip for antibody screening and serum profiling, Mol. Cell. Proteom. 2 (2003) 1342–1349.
[4] K. Büssow, et al., A method for global protein expression and antibody screening on high-density filters of an arrayed cDNA library, Nucleic Acids Res. 26 (1998) 5007–5008.
[5] K. Büssow, E. Nordhoff, C. Lübbert, H. Lehrach, G. Walter, A human cDNA library for high-throughput protein expression screening, Genomics 65 (2000) 1–8.
[6] C. Holz, et al., A human cDNA expression library in yeast enriched for open reading frames, Genome Res. 11 (2001) 1730–1735.
[7] A. Lueking, et al., Protein microarrays for gene expression and antibody screening, Anal. Biochem. 270 (1999) 103–111.
[8] F. Schmidt, et al., Generation of minimal protein identifiers of proteins from two-dimensional gels and recombinant proteins, Electrophoresis 23 (2002) 621–625.
[9] D. Larkin, et al., ICln, a novel integrin aIIbβ3 associated protein, functionally regulates platelet activation, J. Biol. Chem. (2004) (in press).
[10] M.D. Clark, G.D. Panopoulou, D.J. Cahill, K. Bussow, H. Lehrach, Construction and analysis of arrayed cDNA libraries, Methods Enzymol. 303 (1999) 205–233.
[11] J. Greiner, et al., Simultaneous expression of different immunogenic antigens in acute myeloid leukemia, Exp. Hematol. 28 (2000) 1413–1422.
[12] D. Cahill, et al., Bridging genomics and proteomics, in: S. Pennington, M. Dunn (Eds.), Proteomics, BIOS Sci., Oxford, 2001, pp. 1–20.
[13] J. Ohara, W.E. Paul, Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1, Nature 315 (1985) 333–336.
[14] U. Brinkmann, R.E. Mattes, P. Buckel, High-level expression of recombinant genes in *Escherichia coli* is dependent on the availability of the dnaY gene product, Gene 85 (1989) 109–114.
[15] Anon, Colony blot procedure, The QIAexpressionist, 3rd ed., Qiagen, Hilden, Germany, 1997, pp. 31–35.
[16] H. Lehrach, D. Bancroft, E. Maier, Robotics, computing, and biology, Interdiscip. Sci. Rev. 22 (1997) 37–44.
[17] E. Hochuli, H. Dobeli, A. Schacher, New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residues, J. Chromatogr. 411 (1987) 177–184.
[18] W.H. Robinson, et al., Autoantigen microarrays for multiplex characterization of autoantibody responses, Nat. Med. 8 (2002) 295–301.
[19] Z.S. Rahman, et al., A novel susceptibility locus on chromosome 2 in the (New Zealand Black × New Zealand White) F1 hybrid mouse model of systemic lupus erythematosus, J. Immunol. 168 (2002) 3042–3049.
[20] S.F. Altschul, et al., Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.
[21] T. Gaasterland, Structural genomics: bioinformatics in the driver's seat, Nat. Biotechnol. 16 (1998) 625–627.

[22] C.A. Borrebaeck, Antibodies in diagnostics—from immunoassays to protein chips, Immunol. Today 21 (2000) 379–382.
[23] A.D. Steinberg, D.M. Klinman, Pathogenesis of systemic lupus erythematosus, Rheum. Dis. Clin. North Am. 14 (1988) 25–41.
[24] Y. Lim, et al., Identification of autoantibodies associated with systemic lupus erythematosus, Biochem. Biophys. Res. Commun. 295 (2002) 119–124.
[25] E. Feist, et al., Proteasome alpha-type subunit C9 is a primary target of autoantibodies in sera of patients with myositis and systemic lupus erythematosus, J. Exp. Med. 184 (1996) 1313–1318.
[26] J. Arribas, M. Luz Rodriguez, R. Alvarez-Do Forno, J.G. Castano, Autoantibodies against the multicatalytic proteinase in patients with systemic lupus erythematosus, J. Exp. Med. 173 (1991) 423–427.
[27] K. Egerer, et al., Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases, J. Rheumatol. 29 (2002) 2045–2052.
[28] A. Ghirardello, et al., Diagnostic tests for antiribosomal p protein antibodies: a comparative evaluation of immunoblotting and ELISA assays, J. Autoimmun. 19 (2002) 71–77.
[29] L. Caponi, D. Chimenti, F. Pratesi, P. Migliorini, Anti-ribosomal antibodies from lupus patients bind DNA, Clin. Exp. Immunol. 130 (2002) 541–547.
[30] A. Desbos, et al., Autoantibodies directed against ribosomal proteins in systemic lupus erythematosus and rheumatoid arthritis: a comparative study, Autoimmunity 35 (2002) 427–434.
[31] K.M. Murphy, A.B. Heimberger, D.Y. Loh, Induction by antigen of intrathymic apoptosis of $CD4^{+}CD8^{+}TCR^{lo}$ thymocytes in vivo, Science 250 (1990) 1720–1723.
[32] M. Assenmacher, J. Schmitz, A. Radbruch, Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells, Eur. J. Immunol. 24 (1994) 1097–1101.
[33] M. Prat, G. Gribaudo, P.M. Comoglio, G. Cavallo, S. Landolfo, Monoclonal antibodies against murine gamma interferon, Proc. Natl. Acad. Sci. USA 81 (1984) 4515–4519.
[34] H. Zhu, et al., Analysis of yeast protein kinases using protein chips, Nat. Genet. 26 (3) (2000) 283–289.
[35] H. Zhu, et al., Global analysis of protein activities using proteome chips, Science 293 (5537) (2001) 2101–2105.
[36] S. Wiemann, et al., CDNAs for functional genomics and proteomics: the German Consortium, C. R. Biol. 326 (10–11) (2003) 1003–1009.
[37] A.J. Walhout, et al., Protein interaction mapping in *C. elegans* using proteins involved in vulval development, Science 287 (5450) (2000) 116–122.
[38] J. Reboul, et al., *C. elegans* ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression, Nat. Genet. 34 (1) (2003) 35–41.
[39] B. Kersten, et al., Generation of Arabidopsis protein chips for antibody and serum screening, Plant Mol. Biol. 52 (5) (2003) 999–1010.
[40] J.A. Heyman, et al., Genome-scale cloning and expression of individual open reading frames using topoisomerase I-mediated ligation, Genome Res. 9 (4) (1999) 383–392.
[41] A. Lueking, et al., A system for dual protein expression in *Pichia pastoris* and *Escherichia coli*, Protein Expr. Purif. 20 (3) (2000) 372–378.
[42] W.H. Robinson, L. Steinman, P.J. Utz, Protein and peptide array analysis of autoimmune disease, Biotechniques (Suppl) (2002) 66–69.
[43] W.H. Robinson, L. Steinman, P.J. Utz, Protein arrays for autoantibody profiling and fine-specificity mapping, Proteomics 3 (11) (2003) 2077–2084.
[44] S. Ooka, et al., Autoantibodies to low-density-lipoprotein-receptor-related protein 2 (LRP2) in systemic autoimmune diseases, Arthritis Res. Ther. 5 (3) (2003) R174–R180.
[45] V. Corrigall, L.A. O'Neill, Using immunotherapy to treat rheumatic diseases, Trends Immunol. 23 (8) (2002) 383–384.
[46] U. Gubler, B.J. Hoffman, A simple and very efficient method for generating cDNA libraries, Gene 25 (1983) 263–269.