Proteomics-based Identification of Human Acute Leukemia Antigens That Induce Humoral Immune Response*

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The identification of panels of tumor antigens that elicit an antibody response may have utility in cancer screening, diagnosis, and establishing prognosis. Until now, autoimmunity in cancer has been mainly revealed in solid tumors. The aim of this study was to apply the proteomic approach to the identification of proteins that commonly elicit a humoral response in acute leukemia (AL). Sera from 21 newly diagnosed patients with AL, 20 patients with solid tumors, and 22 noncancer controls were analyzed for antibody-based reactivity against AL proteins resolved by two-dimensional electrophoresis. As a result, autoantibody against a protein identified by mass spectrometry as Rho GDP dissociation inhibitor 2 was detected in sera from 15 of 21 patients with AL (71%). By contrast, such antibody was detected in sera from one of 20 patients with solid tumors (5%) and one of 22 noncancer controls (4.5%). Five other protein autoantibodies were also found in AL patients with a high frequency and constituted the major target antigens of the AL autoimmune response. The findings of autoantibodies against Rho GDP dissociation inhibitor 2 and other proteins in sera of patients with AL suggest that the proteomic approach we have implemented may have utility for the development of a serum-based assay for AL screening and diagnosis. *Molecular & Cellular Proteomics* 4: 1718–1724, 2005.

There is substantial evidence for a humoral immune response to cancer in humans as demonstrated by the identification of antibodies against a number of intracellular and surface antigens in patients with various tumor types (1–3). Humoral immune reactivity in many cases coincides with cellular responses, and these targets possibly represent target structures in vaccinations against cancer. Thus, many T-cell antigens have been characterized by a primary serologic identification by recombinant expression cloning approach. In this line of research e.g. the antigens NY-ESO-1 and survivin both elicit humoral as well as cellular immune responses (4, 5). The identification of panels of tumor antigens that elicit an antibody response may have utility in cancer screening or diagnosis or in establishing prognosis. For example, somatic alterations in the p53 gene elicit a humoral response in 15–40% of the patients with tumor, and the detection of anti-p53 antibodies can predate the diagnosis of cancer. The presence of p53 autoantibodies has been observed in 15% of patients with breast cancer and has been shown to be associated with a bad prognosis (6). Tumor regression has been demonstrated in small cell lung carcinoma patients with autoantibodies to onconeural antigens. Autoantibodies to the 90-kDa heat shock protein have also been associated with poor survival in breast cancer (7). The proto-oncogene c-erbB-2/HER2/neu, which encodes a growth factor receptor, is overexpressed in 20–30% of patients with breast cancer. The presence of c-erbB-2/HER2/neu autoantibodies has been observed in 11% of cases and has been found to correlate with poor outcome (8). On the other hand, the presence of MUC1 autoantibodies has been associated with a reduced risk for disease progression in patients with breast cancer. At present, MUC1/CA15-3 is used as a circulating marker for breast cancer (9). The CA15-3 concentration at initial presentation has prognostic significance. Serial measurements have the potential to both detect recurrences preclinically and monitor the treatment of metastatic breast cancer.

Until now, autoimmunity in cancer has been mainly revealed in solid tumors, including lung cancer (10), breast (11, 12), and neuroblastoma (13). There is also some evidence that chronic lymphocytic leukemia (CLL) B-lymphocytes are frequently committed to production of natural autoantibodies, and the occurrence of autoantibodies in CLL is of prognostic relevance (14–16). To investigate whether there is also a humoral

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The abbreviations used are: CLL, chronic lymphocytic leukemia; AL, acute leukemia; ECL, enhanced chemiluminescence; PMF, peptide mass fingerprint; hnRNP, heterogeneous nuclear ribonucleoprotein; GDI, GDP dissociation inhibitor; 2-DE, two-dimensional electrophoresis; CAPZA1, F-actin capping protein; ENO1, α-enolase; PCNA, proliferating cell nuclear antigen.

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immune response to acute leukemia (AL) in humans and whether the autoantibodies are specific to AL, we implemented a proteomic approach to identify proteins that induce an antibody response in patients with AL. We used two-dimensional electrophoresis (2-DE) to simultaneously separate thousands of individual cellular proteins from human AL cells. Separated proteins were transferred onto PVDF membranes, and sera from AL patients and other solid tumor and noncancer controls were screened individually by Western blot analysis for antibodies that react against separated proteins. Proteins that reacted with the subjects’ sera were identified by mass spectrometric analysis. We report in this study that sera from 71% of the AL patients exhibited IgG-based reactivity against proteins identified as GDP dissociation inhibitor (GDI). By contrast, sera from 5% (one of 20) of patients with other solid tumors and 4.5% (one of 22) of the patients with noncancer controls exhibited reactivity against Rho-GDI2, respectively. Five other protein autoantibodies were also found in AL patients with a significantly higher frequency and constituted the major target antigens of the AL autoimmune response.

EXPERIMENTAL PROCEDURES

Cells and Serum Specimens—The leukemic cells were derived from bone marrow aspirates of a group of 21 patients aged 18–64 years (median, 44 years) with AL at the time of initial diagnosis before chemotherapy, and all the patients provided informed consent for the testing. The diagnosis was made according to the French-American-British classification system. Of the 21 cases, there were three cases with M1 type of acute myelocytic leukemia, three cases of M2, four cases of M3, three cases of M4, three cases of M5, and five cases of acute lymphocytic leukemia. Leukemic cells were obtained by Ficoll-Hypaque gradient centrifugation of heparinized bone marrow. The AL cells were pelleted in microcentrifuge tubes and frozen at −80 °C. The purity of the samples submitted to subsequent 2-DE analysis was greater than 90%, which was determined by morphological analysis under microscopy. The sera from 21 patients mentioned above were investigated for the autoantibodies against the leukemia cells. For the control, the sera from 20 patients with solid tumors, including five patients with lung cancer, four with stomach cancer, five with breast cancer, three with colon cancer, and three with liver cancer (all histologically confirmed), were investigated at the time of initial diagnosis before chemotherapy. Sera from 22 healthy volunteers were also investigated.

2-DE and Western Blot—The leukemic cells frozen at −80 °C were lysed in solubilization buffer (8 M urea, 2% CHAPS, 0.5% carrier ampholytes (pH 3–10), 2% DTT) and stored at −80 °C until use. Proteins derived from the extracts of leukemic cells were separated by 2-DE as described previously (17), and the separated proteins were transferred onto a PVDF membrane (Amersham Biosciences). Protein patterns in some gels were visualized directly by silver staining and, for some membranes, by Coomassie Blue staining. The 2-DE images were captured using ImageScanner (Amersham Biosciences). Spot detection, quantification, and alignment were performed with the ImageMaster 2D Elite 3.10 software (Nonlinear Dynamics Ltd.). For hybridization with serum, unstained PVDF membranes were incubated with a blocking buffer consisting of Tris-buffered saline, 5% nonfat dry milk, and 0.1% Tween 20 for 2 h and then were washed and incubated either with serum obtained from patients or with normal control serum at a 1:100 dilution for 1 h at room temperature. After three washes with washing buffer (Tris-buffered saline containing 0.1% Tween 20), the membranes were incubated with a secondary antibody (horseradish peroxidase-conjugated anti-human IgG antibody) at a 1:10,000 dilution for 30 min at room temperature, washed, and briefly incubated with enhanced chemiluminescence (ECL) reagents (Amersham Biosciences).

Protein Identification—After the Western blot and film exposure to the PVDF membrane incubated with ECL, we washed the PVDF membrane with the washing buffer and stained the PVDF membrane with Coomassie Brilliant Blue. The profile of the proteins on the membrane was the same as that of the map of 2-DE (the proteins on the 2-DE gel were not transferred onto the PVDF membrane) visualized by silver staining or Coomassie Brilliant Blue staining. We put the film exposed to the PVDF membrane incubated with the ECL reagents onto the stained PVDF membrane to localize the exposed spot on the PVDF membrane. We matched the spots on the PVDF membrane with the 2-DE map of the same sample. The identified protein spots from the 2-DE gel stained by Coomassie Brilliant Blue. For protein identification by mass spectrometry, excised proteins were digested as described previously (18). Protein identification was repeated at least twice using spots from different gels. The obtained peptide mass fingerprint (PMF) was used to search through the Swiss-Prot and National Center for Biotechnology Information nonredundant (NCBI) databases by the Mascot search engine (www.matrixscience.co.uk). The protein identification was reconfirmed by an ESI-MS/MS approach. The database search was finished with the Mascot search engine (www.matrixscience.co.uk) using a Mascot MS/MS ion search. In addition, the amino acid sequences of the peptides were deduced with the peptide sequencing program MassSeq.

Immunoprecipitation and Western Blot Analysis—For immunoprecipitation experiments, cells were lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 1% Nonidet P-40, 1 mM EDTA) containing a mixture of protease inhibitors, and whole cell extracts were centrifuged at 12,000 rpm for 20 min to remove cell debris. Immunoprecipitations were performed by incubating whole cell extracts with the indicated antibody, preincubated with protein A/G-Sepharose (Santa Cruz Biotechnology), while rocking at 4 °C overnight. Immunoprecipitates were washed three times with buffer, resuspended in 40 μl of 1× SDS sample buffer, and then resolved by SDS-PAGE. For Western blot analysis, cells were lysed in lysis buffer supplemented with a mixture of protease inhibitors. Whole lysates were resolved by SDS-PAGE and subsequently transferred onto nitrocellulose (Amersham Biosciences) and probed with the primary antibody after blocking. Membranes were developed using the ECL method (Amersham Biosciences).

RESULTS

MS Identification of Rho-GDI2 and Other Autoantigens—The identity of this set of autoantigens was determined by MALDI-TOF MS after trypsin digestion (Table I). The A3 spot was first identified as Rho-GDI2 (Fig. 1A) by PMF using MALDI-TOF MS (Fig. 2A). Image analysis on the 2-DE maps showed that there was no obvious difference in Rho-GDI2 expression between AL cells and normal white blood cells (Fig. 1C). After database searching with PMF, the protein score was significant (p < 0.05), and the sequence coverage (matched peptides) reached 61% with a difference between measured and calculated masses below 0.1 Da. The analysis of PMF was made for at least three samples from three independent experiments. Furthermore six peptides chosen from the PMF were subjected to a sequence analysis by
nano-ESI-MS/MS, and data from one representative experiment are shown in Fig. 2B; results were consistent with the analysis by PMF. The identification for other spots of autoantigens was also made according to the same methods (Table I).

Reactivity of Sera from AL Patients with Their Autologous Proteins—Sera obtained at the time of diagnosis from 21 patients with AL were investigated for the presence of IgG antibody-based reactivity against their autologous AL proteins resolved by 2-DE (Fig. 1B). Sera from 15 of 21 patients with AL (Table II), including two sera from patients with M1 type, two with M2, three with M3, three with M4, two with M5, and three with acute lymphocytic leukemia, exhibited IgG-based reactivity against a protein identified by mass spectrometry as Rho-GDI2 (Fig. 2C). Positive sera were reactive against Rho-GDI2 at the highest serum dilution tested, which was 1:1000. The French-American-British classification information of AL suggested that the occurrence of autoantibodies against Rho-GDI2 was a common feature of the different AL types listed above. The AL specificity of Rho-GDI2 autoantibodies was determined by screening sera from 20 patients with other types of cancer and 22 sera from noncancer controls (Table II). Only one serum in the cancer control group, from a patient with liver cancer, exhibited immunoreactivity against Rho-GDI2. The noncancer control group consisted of sera from 22 healthy subjects, and only one serum exhibited immunoreactivity against Rho-GDI2.

Autoantibodies against γ-actin and F-actin capping protein (CAPZA1) identified for other protein spots (A5 and A9) and recognized by the sera of AL patients also showed AL specificity (Table I). Sera from 10 of 21 patients with AL exhibited IgG-based reactivity against γ-actin and CAPZA1. By contrast, one serum sample in the cancer control group and two serum samples in the noncancer control group exhibited immunoreactivity against γ-actin and CAPZA1. Two autoantigens identified as heterogeneous nuclear ribonucleoprotein L (hnRNP L) and tubulin-α 6 were found at a higher frequency for eliciting humoral immune responses in patients of both the

| Spot | Protein name | accession no. | NCBInr | Mₚ | pI | Sequence | coverage | Score |
|------|--------------|---------------|--------|-----|----|----------|----------|-------|
| A1   | ENO1 protein | gi|29792061 | 47,139 | 48,978 | 7.01 | 7.05 | 54 | 172 |
| A2   | ENO1 protein | gi|29792061 | 47,139 | 48,978 | 7.01 | 6.79 | 61 | 192 |
| A3   | Rho-GDI1 (Rho-GDI2) | gi|10835002 | 22,974 | 26,645 | 5.10 | 5.11 | 61 | 104 |
| A4   | Heterogeneous nuclear ribonucleoprotein | gi|46812638 | 60,195 | 66,078 | 6.65 | 7.12 | 43 | 198 |
| A5   | CAPZA1 protein | gi|12652785 | 32,902 | 37,210 | 5.45 | 5.31 | 57 | 132 |
| A6   | PCNA | gi|33233451 | 28,750 | 32,465 | 4.57 | 4.49 | 45 | 115 |
| A7   | Prohibitin | gi|46360168 | 29,802 | 34,756 | 5.57 | 5.38 | 39 | 112 |
| A8   | Tubulin-α 6 | gi|14389309 | 49,863 | 51,253 | 4.96 | 4.79 | 32 | 128 |
| A9   | γ-Actin | gi|178045 | 25,862 | 30,616 | 5.65 | 5.41 | 67 | 100 |

The calculation of experimental pI and Mₚ was based on migration of the protein spot on 2-DE gels. The scores greater than 63 are significant (p < 0.05). Theor., theoretical; Observ., observed.

**Fig. 1.** Screening of autoantibodies in acute leukemia. A, silver staining of acute leukemia proteins separated by two-dimensional PAGE showing protein spots (A1–A9) recognized by sera from patients with acute leukemia. B, close-up sections showing one representative result of Western blot performed with AL patient serum against AL cells. C, close-up sections of specific two-dimensional gel regions for three representative acute leukemic cells and three representative normal white blood cells. The circled regions show the protein of Rho-GDI2 identified by MS probe.
AL and solid tumor groups compared with healthy volunteers. \( \text{H9251} \)-Enolase (ENO1) showed no significant difference among the three groups. There was no correlation between the status of patients with AL and the occurrence of autoantibodies. The occurrence of autoantibodies was not correlated with the age and sex of the patient.

**Validation of the Occurrence of Rho-GDI2 Autoantibodies in Serum from AL Patients**—In our study we demonstrated by two-dimensional PAGE and Western blot analyses that 71% of AL sera contained autoantibodies against Rho-GDI2. As the common occurrence of Rho-GDI2 autoantibody may represent an important avenue for cancer diagnosis, we did another type of experiment to validate the finding. Western blot analysis of proteins immunoprecipitated from AL cells by anti-Rho-GDI2 antibody and separated by one-dimensional PAGE was performed with either AL patient sera or normal control sera. Whole cell extracts were immunoprecipitated with an anti-Rho-GDI2 antibody, and equal amounts of the immunoprecipitated protein were used for immunoblot analysis with AL patient sera (Fig. 3A), normal control sera (Fig. 3B), and anti-Rho-GDI2 antibody (Fig. 3C). As results, 11 sera from 21 patients with AL showed positive reactivity against Rho-GDI2, and none of the sera from healthy volunteers showed the reactivity against Rho-GDI2.

**DISCUSSION**

Rho family members are small GTPases that are known to regulate malignant transformation and motility of cancer cells. The increase in the amount of Rho GTPases appears to be a frequent event in different types of human tumors (19) and may contribute to the aggressive nature of tumor cells as well as to tumor-induced biological processes involved in disease progression (20–22). The activities of Rho are regulated by Rho-GDIls, which form a complex with the GDP-bound form of the Rho family of GTPases and inhibit their activation. Rho-GDI has been implicated in tumor cell apoptosis, invasion, and metastases. Reduced expression of Rho-GDI in breast tumor tissues are correlated with the nodal involvement and metastasis. Rho-GDI2 mRNA is expressed in less invasive T24 tumor cells and not in highly invasive T24T cells (23). In ovarian tumors, Rho-GDI2 is up-regulated (24). Our previous study showed that Rho-GDI\( \text{H9251} \) is overexpressed in the AL cells (25). The different expression of Rho-GDI2 in various cancers may imply that Rho-GDI2 is a potential prognostic factor. The mechanism by which Rho-GDI2 elicits autoantibody and the clinical significance of the autoantibody in AL patients remain unknown. However, the significantly high frequency of autoantibody against Rho-GDI2 in AL patients implied its potentiality in clinical settings and was also a starting point to further our study. Immunogenicity may depend on the level of expression, posttranslational modification, or other types of protein processing, the extent of which may be variable among tumors of a similar type. Rho-GDI2 is known to undergo posttranslational modification and is predominantly expressed in hematopoietic cells, including B- and T-lymphocyte cell lines, and also expressed in breast tissues (26, 27). Prior studies have also shown that Rho-GDI2 is implicated in the regulation of lymphocyte survival (28). In addition, the expression of Rho-GDI2 is dramatically increased during proliferation and differentiation in vitro of hematopoietic precur-

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**Fig. 2.** Identification of protein spot A3 recognized by sera from patients with acute leukemia by mass spectrometry. A, identification of Rho-GDI2 by MALDI-TOF MS after trypsin digestion of the protein spot A3. Indicates the modification of oxidation at a Met residue. \# indicates the modification of carbamidomethyl at a Cys residue. B, the peptide of 966.50 chosen from the PMF for the A3 spot was sequenced by nano-ESI-MS/MS. C, close-up sections of Western blots showing IgG-based reactivity of Rho-GDI2 protein with a serum sample from acute leukemia or healthy control.
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TABLE II
Rho-GDI2 and other autoantibodies in subject sera

| Positive autoantibodies in subject sera | Number of subjects |
|----------------------------------------|-------------------|
|                                        | Rho-GDI2 | ENO1 | hnRNP L | CAPZA1 | PCNA | Tubulin-α 6 | γ-Actin |
| Acute leukemia                         | 21       | 15 (71%) | 18 (86%) | 16 (76%) | 10 (48%) | 3 (14%) | 6 (29%) | 10 (48%) |
| Solid tumor                            | 20       | 1 (5%)   | 16 (80%) | 14 (70%) | 1 (5%)  | 0 (0%)  | 7 (35%) | 1 (5%)  |
| Healthy control                        | 22       | 1 (4.5%) | 20 (90%) | 3 (14%)  | 2 (9%)  | 0 (0%)  | 0 (0%)  | 2 (9%)  |

Fig. 3. Validation of Rho-GDI2 autoantibody with immunoprecipitation and Western blot. Western blot analysis of proteins, immunoprecipitated (IP) from AL cells by anti-Rho-GDI2 antibody and separated by one-dimensional PAGE, were performed with either AL patient sera or normal control sera. Whole cell extracts were immunoprecipitated with an anti-Rho-GDI2 antibody, and equal amounts of the immunoprecipitated protein were used for immunoblot analysis with AL patient sera (A), normal control sera (B), and anti-Rho-GDI2 antibody (C).

We also detected several other proteins against which sera from patients with AL exhibited immunoactivity in Western blots. These proteins (Table II) constituted the major target antigens of the AL autoimmune response and defined its characteristic pattern. Among them, γ-actin and CAPZA1 are key components of the eukaryotic cytoskeleton that can regulate biophysical characteristics and cellular signaling of the circulating cells and are involved in early steps during metastasis formation in vivo and in vitro (30). In a recent study, Prasannan et al. (13) found that sera from nearly 50% of patients with neuroblastoma but none of the other solid tumors and other controls exhibited IgG-based reactivity against β-tubulin I and III isoforms, another cytoskeletal component with an estimated M_r of 50,000, identified by NH2-terminal sequence and mass spectrometric analysis. In a study of various cell lines, a prepared mouse monoclonal antibody of β-tubulin III reacted only with a neuroblastoma cell line, and there was no reactivity among a large number of nonneuronal tumor types. Given the specificity of certain β-tubulin isoforms to tumors of neuroectodermal origin, the occurrence of autoantibodies to specific tubulin isoforms could be a useful diagnostic marker (31). In CLL, a subset of B-cells expressing the CD5 marker, a 67-kDa molecule, has been implicated in the pathogenesis of the programming involved in the secretion of autoantibodies against cytoskeletal components (32). Neuroblastoma patient sera that reacted with β-tubulin I and III isoforms in neuroblastoma tissues did not react with β-tubulin I and III isoforms found in normal brain tissue. The occurrence of β-tubulin in neuroblastoma as immunogen may have utility in diagnosis or immunotherapy of this aggressive childhood tumor (13). Our results also revealed the similar findings that sera from AL patients that reacted with γ-actin and F-actin capping protein in AL did not react with them found in normal mononuclear cells. It suggests that the occurrences of γ-actin and F-actin capping proteins in AL patients are immunogenic.

Rho-GDI2, CAPZA1, proliferating cell nuclear antigen (PCNA), and γ-actin were previously unknown to be the targets of autoantibodies produced in human with or without specific autoimmune diseases. For two other identified proteins, hnRNP L and ENO1, autoantibodies against them have already been described in various pathological situations, mainly in nonorgan-specific autoimmune diseases (33–35). hnRNP L belongs to the large superfammy of hnRNPs, which are frequently recognized by autoantibodies present in patients with nonorgan-specific autoimmune diseases; antibodies to hnRNP A1, A2, and B have been previously found in patients with rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue diseases, and antibodies directed against hnRNP L (polypyrimidine tract-binding protein) were detected in the sera of systemic sclerosis patients (36, 37). Notably the amino acid sequence of hnRNP L is highly homologous to that of hnRNP I; together they constitute a new family of hnRNP proteins within the ribonucleoprotein consensus RNA-binding proteins. Three patients with AL developed autoantibody against PCNA, a proliferation-related component of the enzyme complex used to synthesize DNA, which was found to be overexpressed in AL cells compared with normal cells in our study (data not shown).

The mechanism for the development of autoantibodies against certain proteins in cancer is not very clear. One key
issue to understanding the mechanism of AL autoimmunity is to determine what characteristics the self-antigens, such as those identified in this study, may share. In this regard, several studies have indicated that apoptotic cells could constitute a reservoir of self-antigens that elicit an autoimmune response in cancer and autoimmune diseases and contribute to the development of autoimmunity (38). Indeed apoptosis is important for the preservation of peripheral blood cell homeostasis, and interesting results have revealed the capacity of Rho-GDI2, specifically expressed in hematopoietic tissues and predominately in B- and T-lymphocyte cell lines, to be proteolyzed by caspase proteases in apoptosis. In MG-132 and Fas-induced apoptosis in Mo7e leukemic cells and Jurkat T cells, Rho-GDI2 underwent posttranslational modifications during apoptosis, such as cleavage by caspases-3, and was rapidly truncated to a 23,000 M_r fragment (39, 40). Other autoantigens identified in this study were also known to be involved in apoptosis and are relocated to the surface of apoptotic blebs or vesicles released by cells that enter a process of programmed death. Changes in actin dynamics, i.e. the rate of actin polymerization and depolymerization, modulate the transduction of the apoptotic signal-committing lymphocytes, withdrawn from required growth factors, to the death pathway, and a disruption in the regulation of actin dynamics, not simply F-actin stabilization, was required to affect the transduction of an apoptotic signal (41). PCNA can regulate apoptosis through its interaction with ML1 myeloid cell leukemia 1, a Bcl-2 homologue (42). Deactivation of regulatory proteins hnRNP A1 and A2 during SC-1 can induce apoptosis (43). It should be noted that these six proteins can undergo modification during apoptosis that may confer the ability to induce autoantibody production in AL patients.

This study showed that combining 2-DE and mass spectrometry constitutes a powerful tool to identify the whole autoantibody pattern of AL. Although the data are somewhat preliminary, it is of interest to perform further studies on the antigens detected in this study that might be candidates as diagnostic markers and therapy targets in AL. We considered the complexity of the autoantibodies in our research. We did use the sera from autoimmune patients (four cases), patients with other hematologic disorders (anemia and lymphoma, six cases), and patients with acute and chronic inflammation (five cases), and patients with acute and chronic inflammation (five cases) to react with the AL cell proteins separated by 2-DE. There were various antibodies that could react with the AL cells in the sera of the above patients. However, the autoantibodies that showed high frequency in the sera of AL patients were not found in the sera of the above patients. It is necessary to collect more samples to identify the relative specific AL-associated autoantibodies and the specific antigens recognized by these autoantibodies. Our long term goal is to link information on autoantibody status (i) to achieve a serum marker for AL early diagnosis, (ii) as a predictor of AL progression, and (iii) as a guide to select the appropriate treatment therapy. In addition to their direct clinical application, autoantibodies found in AL patients could aid in the discovery of novel oncogenic proteins that are involved in the pathophysiology of AL and also facilitate the discovery of specific antigen in AL, which will lay the basis for the specific therapy for AL.

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