Increased Plasma Concentrations of Cytoskeletal and Ca\(^{2+}\)-Binding Proteins and Their Peptides in Psoriasis Patients

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BACKGROUND: The mechanisms underlying psoriatic pathogenesis are not fully understood and might be elucidated by identifying novel disease-related molecular markers, including autoantigens.

METHODS: We used 2 proteomic methods to analyze plasma samples from 20 psoriasis patients and 20 matched healthy donors. The first method focused on evaluating changes in glycoprotein concentrations and the plasma proteome, and the second method assessed endogenous proteolytic activity by analyzing the low molecular weight component of plasma.

RESULTS: The integrated proteomic and peptidomic analysis identified a number of proteins and their fragments present at different concentrations in the plasma of psoriasis patients and healthy donors. We used ELISA to independently verify the changes in the concentrations of several of these proteins. One intriguing finding, increased concentrations of cytoskeletal and actin-binding proteins and their peptides in psoriatic plasma, suggested disease-related cell leakage of these proteins and their increased proteolysis. Among the increased proteins and peptides were thymosin \(\beta 4\), talin 1, actin \(\gamma\), filamin, and profilin. Increased concentrations of Ca\(^{2+}\)-binding proteins calgranulins A and B in psoriatic plasma were also observed, confirming previous reports, and appeared to be relevant to the increase of cytoskeletal components. Another notable change in psoriatic plasma was a striking decrease in fibrinogen fragments.

CONCLUSIONS: The identified increased concentrations of cytoskeletal proteins, their peptides, and calgranulins in psoriatic plasma, as well as the underlying altered protease activity, are proposed to be related to psoriasis pathogenesis.

Psoriasis is a common inflammatory disease of the skin with a poorly understood pathogenesis (1, 2). It is characterized by inflamed, thickened, and scaly skin that shows hyperproliferation and altered differentiation of keratinocytes, inflammation, and angiogenesis. Psoriasis affects 2%–3% of the Caucasian population and frequently develops in early adulthood (3). Both a genetic predisposition and environmental factors such as skin injury, stress, infections, and use of certain drugs are thought to play roles in the development and progression of the disease. The genetic basis of psoriasis is complex, with at least 9 susceptibility loci having been identified. Psoriasis exhibits many features of a T cell–mediated autoimmune disease, although the autoantigens involved in the generation of the immune response remain unknown. Complex cytokine signaling and interaction between epidermal keratinocytes and mononuclear leukocytes are thought to play major roles in the pathogenesis of psoriasis (1, 4). The severity of the disease is evaluated by the percentage of the body surface area affected or by the Psoriasis Area and Severity Index.

There is no specific need for improving diagnostic methods for psoriasis because the disease is easily diagnosed from the patient’s symptoms; however, there are clear needs for a better understanding of disease pathogenesis, predicting the future severity of psoriasis or the development of psoriatic arthritis, identifying new therapeutic targets, and better patient stratification for existing therapies. To address some of these issues and to possibly obtain clues as to the nature of potential autoantigens, we performed a study aimed at identifying plasma markers associated with psoriasis.

In recent years, proteomic analysis has led to the identification of candidate biomarkers for a variety of physiological and pathologic conditions (5–7). In our study, we used a combination of 2 proteomic methods to analyze both the protein content and low molecular
weight (LMW)$^3$ peptide content of plasma samples from psoriasis patients and healthy donors. The first method consisted of removing the abundant plasma proteins, multilectin affinity chromatography (M-LAC), trypsin digestion of the fractionated proteins, and analysis with nanoflow liquid chromatography coupled with mass spectrometry (nano-LC-MS/MS) (8, 9). We have previously shown M-LAC to be an effective method for enriching glycoproteins present at low concentrations in serum or plasma and attribute its usefulness to the combination of specificities of the 3 lectins selected to bind major glycosylation classes of plasma proteins.

The second method used ultracentrifugation and analysis of peptides by nano-LC-MS/MS with high-accuracy Fourier transform mass spectrometry to evaluate the LMW fraction of plasma (10). The LMW peptidome is a rich source of biological information. Peptides present in blood samples are thought to be fragments of proteins that have been partially digested by proteases, either in tissues in vivo or in blood ex vivo following blood collection (11–13). The key element of the intact peptidome method we used is our omission of the traditional enzymatic-digestion step, which allowed assessment of endogenous proteolytic activity.

Materials and Methods

PLASMA SAMPLES

We obtained plasma samples from Bioreclamation Inc. Twenty samples were from healthy donors (control group of 12 women and 8 men, all Caucasian; age range, 23–69 years; mean, 40.5 years), and 20 plasma samples were from individuals with moderate to severe psoriasis (>5% of body surface area affected; 11 women and 9 men, all Caucasian; age range 23–60 years; mean, 40.8 years). For details, refer to the Supplemental Data file in the Data Supplement that accompanies the online version of this article at http://wwwclinchemorg/content/vol54/issue11. To facilitate proper comparative analysis, we selected donors so that both groups had the same distribution of age, sex, and race. In addition, samples were collected in a uniform manner by strict adherence to established procedures to minimize the effect of sample collection and storage on results. Ten milliliters of blood were collected by venipuncture into BD Diagnostics Vacutainer® tubes containing citrate and centrifuged at 3000g for 10 min. The plasma was removed and frozen within 1 h of blood collection. Samples were stored at −70 °C and underwent not more than 2 freeze/thaw cycles.

REAGENTS

POROS® Protein G (0.2 mL) and POROS anti-HSA (2.0 mL) cartridges were purchased from Applied Biosystems. We obtained 2-mL Seppro™ MIXED12-LC2 columns (currently sold as ProteomeLab™ IgY-12) from GenWay Biotech. Concanavalin A, wheat germ agglutinin, and jacalin were from Vector Laboratories. We obtained sequencing-grade modified trypsin from Promega, BCA Protein Assay Kits and Zeba™ spin columns from Pierce, Centricor and Microcon centrifugal filters from Millipore, and Discovery® BIO C18 cartridges (C18, 2 cm × 4.00 mm, 3-μm particle diameter) from Supelco. ELISA kits for galectin 3 and calprotectin were from Bender MedSystems and Hycult Biotechnology, respectively. ELISA kits for thymosin β 4 (Thb4) and rabbit anti-Thb4 polyclonal antibody were from ALPCO Diagnostics. For chromatography, we purchased HPLC-grade reagents from Fisher Scientific and obtained bovine fetuin and all other chemicals from Sigma–Aldrich.

PROTEOMIC ANALYSIS

Proteomic analysis of plasma samples was performed as previously described (9), with minor modifications. In brief, plasma samples were spiked with bovine fetuin as an internal standard during protein quantification, depleted of abundant proteins, and then fractionated with M-LAC. After M-LAC fractionation, we digested both the nonbound and bound fractions of each sample with trypsin and analyzed the resulting peptides by nano-LC-MS/MS in the data-dependent mode. Peptide sequences and proteins were identified by searching all tandem mass spectrometry spectra against theoretical fragmentation spectra of a human protein database (SwissProt). Further details are provided in the Supplemental Data file in the online Data Supplement.

PEPTIDOMIC ANALYSIS

Peptidomic analysis of plasma samples was performed as previously described (10), with several modifications. In brief, we diluted plasma samples with 9 volumes of 200 mL/L acetonitrile, incubated at ambient temperature for 10 min, centrifuged the diluted plasma samples at 10 000g for 10 min, transferred the supernatant to a prewashed Microcon centrifugal filter with a 10-kDa cutoff, and centrifuged at 3000g for 30 min at 20 °C. We then desalted the LMW component of the plasma samples with Discovery BIO C18 reversed-phase cartridges and analyzed the peptides by nano-LC-MS/MS interfaced with an LTQ FT™ mass spec-

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$^3$ Nonstandard abbreviations: LMW, low molecular weight; M-LAC, multilectin affinity chromatography; nano-LC-MS/MS, nanoflow liquid chromatography coupled with mass spectrometry; Thb4, thymosin β 4.
Cytoskeletal and Ca\textsuperscript{2+}-Binding Proteins in Psoriasis

We analyzed the same 40 samples for changes in the LMW plasma peptidome and identified a number of peptides present at different concentrations in psoriatic and control plasma (Table 2). These peptides were derived from 17 proteins belonging to some of the same protein classes as identified in the proteomic survey, including lipoproteins, cytoskeletal proteins, and fibrinogen. Numerous peptides have been identified for several of the proteins, including fibrinogen, Thb4, filamin-A, and talin 1. These peptides included so-called peptide ladders, which consist of peptides that originate from the same larger peptide and differ by one to several amino acid residues cleaved from either the N-terminal or C-terminal end of the larger peptide (10, 22–24). Examples of the peptide ladders we observed are provided in Table 4 of the online Data Supplement.

CYTOSKELETAL PROTEINS
Our attention was drawn to the cytoskeletal protein group because of the unexpected presence of several members of this group in psoriatic plasma and their virtual absence in the plasma samples from control individuals. The source of the cytoskeletal proteins and their fragments in the circulation of psoriasis patients is unclear. They may have been released by damaged or dying cells, such as keratinocytes or lymphocytes, or they may be derived from platelets, whose integrity is known to be impaired in psoriasis (25), because some of the identified proteins are abundant in platelets.

Both protein and peptide concentrations for actin, filamin, and talin 1 were increased in plasma samples from psoriasis patients compared with the controls (Fig. 1). This finding may be explained by the detection of different stages of protein processing: The proteomic analysis detects intact proteins or their large fragments leaking from cells, whereas the peptidomic method detects further-digested smaller protein fragments. This hypothesis is supported by the fact that the proteomic and peptidomic methods identify fragments scattered throughout the protein sequence, as is shown for talin 1 in Fig. 1 in the online Data Supplement.

Interestingly, the plasma concentrations of certain protein-derived peptides were increased much more than others. For example, the plasma concentration of N-terminal peptide AcSDKPDMAEIKFDSKLLK from Thb4 was 2.2-fold higher in psoriasis patients than in plasma from control individuals (all numbers are ratios of median concentrations), whereas one of the C-terminal peptides (E.KNPLPSKETIQEKQAGES) was increased 390-fold (Fig. 2A). (The 2 periods in the peptide amino acid sequences indicate the beginning and end of the identified peptide. Letters before and after a period indicate the amino acid residues immediately preceding and following the identified peptides, respectively. The absence of a period at one of the ends indicates the N or C terminus of the protein. Ac, acetylated N-terminal amino acid residue.) The concentration of talin 1 peptide S.PEPPAKTSTPEDFIR.M was increased 13-fold, whereas the concentration of peptide A.SNPEFSSIPAQSPEGRA was only 4-fold higher (Fig. 2B), an increase similar to that of talin 1 measured by proteomic analysis. The increased con-
centrations of certain peptides suggest increased proteolytic activity directed toward particular sites on a protein. It is also possible that peptides that are present at the most increased concentrations in plasma are bound to large carrier molecules that protect them from being rapidly cleared from the circulation. The significance of the fact that certain peptides are increased more than others requires further investigation. Such experiments may include mapping cleavage sites to the specificities of certain proteases, identifying peptide-carrier molecules, and investigating the potential existence of autoantibodies against highly increased proteolytic peptides.

Table 1. Proteins identified to be present at different concentrations in the plasma of psoriasis patients and healthy control individuals.

<table>
<thead>
<tr>
<th>Protein Group</th>
<th>Protein</th>
<th>SwissProt accession no.</th>
<th>M-LAC fraction</th>
<th>Median number of spectral countsa</th>
<th>P/Cb ratio of median concentrations based on peak areas of EIC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td>Galectin 3–binding protein</td>
<td>Q08380</td>
<td>Bound</td>
<td>12</td>
<td>3.1 0.001</td>
<td></td>
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<tr>
<td></td>
<td>Galectin 3</td>
<td>P17931</td>
<td>NA</td>
<td>NA NA NA</td>
<td>2.1 0.001</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ binding</td>
<td>Calgranulin A</td>
<td>P05109</td>
<td>Nonbound</td>
<td>3 0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calgranulin B</td>
<td>P06702</td>
<td>Nonbound</td>
<td>4 1</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calprotectin</td>
<td>P05109/P06702</td>
<td>NA</td>
<td>NA NA NA</td>
<td>1.6 0.009</td>
<td></td>
</tr>
<tr>
<td>Transporter (lipoproteins)</td>
<td>Apolipoprotein AI</td>
<td>P02647</td>
<td>Bound</td>
<td>46 25</td>
<td>2.6 0.028</td>
<td></td>
</tr>
<tr>
<td>Complement</td>
<td>Complement C5</td>
<td>P01031</td>
<td>Bound</td>
<td>15 10</td>
<td>3.3 0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complement B</td>
<td>P00751</td>
<td>Bound</td>
<td>48 29</td>
<td>2.1 0.004</td>
<td></td>
</tr>
<tr>
<td>Protease/protease inhibitors</td>
<td>Carboxypeptidase N</td>
<td>P22792</td>
<td>Bound</td>
<td>9 5</td>
<td>3.1 0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparin cofactor 2</td>
<td>P05546</td>
<td>Bound</td>
<td>18 12</td>
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<tr>
<td>Cytoskeletal</td>
<td>Actin γ, cytoplasmic</td>
<td>P63261</td>
<td>Nonbound</td>
<td>5 0</td>
<td>3.5 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aczonin</td>
<td>Q9Y6V0</td>
<td>Nonbound</td>
<td>4 0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calfesmon</td>
<td>Q05682</td>
<td>Nonbound</td>
<td>3 0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Filamin-A</td>
<td>P21333</td>
<td>Nonbound</td>
<td>2 0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MARK 3</td>
<td>P27448</td>
<td>Nonbound</td>
<td>3 0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microtubule-actin cross-linking factor 1, isoform 4</td>
<td>Q96PK2</td>
<td>Nonbound</td>
<td>3 0</td>
<td>1.01</td>
<td>—</td>
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<tr>
<td></td>
<td>Nebulin</td>
<td>P20929</td>
<td>Nonbound</td>
<td>3 0</td>
<td>0.003</td>
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<tr>
<td></td>
<td>PDZ and LIM domain protein 1</td>
<td>O00151</td>
<td>Nonbound</td>
<td>2 0</td>
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</tr>
<tr>
<td></td>
<td>Profilin</td>
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<td>Nonbound</td>
<td>3 0</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Talin 1</td>
<td>Q9Y490</td>
<td>Nonbound</td>
<td>4 0</td>
<td>4.2 0.001</td>
<td></td>
</tr>
</tbody>
</table>

a Rounded to whole numbers.
b P, psoriasis; C, control; EIC, extracted ion chromatogram; NA, data not applicable; MARK 3, microtubule affinity-regulating kinase 3.
c Based on ELISA measurements.
d ELISA results for calprotectin (calgranulin A/calgranulin B heterocomplex).

THYMOSIN B 4
Thb4, a protein from the cytoskeletal/actin-binding group of proteins, demonstrated the greatest increase in proteolytic peptides in plasma from psoriasis patients compared with controls. To confirm our findings and to correlate the increases in Thb4-derived peptide concentrations to changes in intact Thb4 concentrations, we used 2 ELISAs to measure Thb4 in the same set of 40 samples. One of the methods is specific for full-length Thb4, and the other is specific for the Thb4 N terminus. No commercially available antibody specific for the Thb4 C terminus worked well in the ELISA format. The Thb4 concentration as measured by
ELISA with N-terminal specificity was increased 1.8-fold in the plasma samples from psoriasis patients (Fig. 3A). This increase is comparable to the 2.2-fold increase in N-terminal peptides measured with peptidomic analysis. The plasma Thb4 concentration measured by the ELISA specific for full-length Thb4 was 4.9-fold higher in psoriasis patients than in controls. This ELISA may also have detected some Thb4 fragments but not highly increased C-terminal peptides.

**Ca²⁺-BINDING PROTEINS**

The concentrations of calgranulins A and B as assessed by proteomic analysis were increased in psoriatic plasma compared with the controls (Fig. 3B). We also measured the plasma concentration of calprotectin (a heterodimer of calgranulins A and B) by ELISA (ratio of median concentrations, 2.0; Table 1 and Fig. 3B) to confirm the proteomic results. These findings support earlier reports of an increase in the calgranulin concentration in the circulation of psoriasis patients.

**FIBRINOGEN**

Decreased concentrations of peptides originating from fibrinogen chains Aα and Bβ were observed in psoriasis patient plasma (Fig. 4). It is possible that these decreases are related to the increase in fibrinogen concentrations reported to occur during acute-phase conditions and in inflammatory diseases (26, 27) and may be a consequence of the decreased physiological proteolysis of fibrinogen due to reduced cleavage by plasmin and other proteases. During the proteomic analysis, we also observed an increase in fibrinogen Aα and Bβ concentrations in plasma samples from psoriasis patients; however, the increase was below the established cutoff value of 2 for the ratio of the median protein concentrations. Because posttranslational modifications such as phosphorylation, glycosylation, and deimination (citrullination) are thought to protect fibrinogen from proteolysis (27), increases in fibrinogen modifications may be the cause of the observed decreased fibrinogen proteolysis (see Figs. 2 and 3 in the online Data Supplement).

**Discussion**

Considering the results of our proteomic and peptidomic analysis and the known functions of calgranulins, we hypothesize that it is important that the observed increase in the plasma concentrations of calgranulins A and B in psoriasis patients coincides with the rise in the

### Table 2. Proteins whose fragments were identified to be present at different concentrations in the plasma of psoriasis patients and healthy control individuals.

<table>
<thead>
<tr>
<th>Protein Group</th>
<th>Protein</th>
<th>SwissProt accession no.</th>
<th>Median number of spectral countsa</th>
<th>Psoriasis</th>
<th>Control</th>
<th>P</th>
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</thead>
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<td>Transporter (lipoproteins)</td>
<td>Apolipoprotein AIV</td>
<td>P06727</td>
<td>0</td>
<td>3</td>
<td>0.0004</td>
<td></td>
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<tr>
<td></td>
<td>Apolipoprotein CIII</td>
<td>P02656</td>
<td>0</td>
<td>2</td>
<td>0.0011</td>
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<td>Coagulation</td>
<td>Fibrinogen Aα</td>
<td>P02671</td>
<td>34</td>
<td>160</td>
<td>0.0001</td>
<td></td>
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<tr>
<td></td>
<td>Fibrinogen Bβ</td>
<td>P02675</td>
<td>3</td>
<td>13</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Cytoskeletal</td>
<td>Actin γ, cytoplasmic</td>
<td>P63261</td>
<td>7</td>
<td>1</td>
<td>0.0012</td>
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</tr>
<tr>
<td></td>
<td>Actin α 1, skeletal</td>
<td>P68133</td>
<td>3</td>
<td>0</td>
<td>0.002</td>
<td></td>
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<tr>
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<td>Caldesmon</td>
<td>Q05682</td>
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<td>0.003</td>
<td></td>
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<tr>
<td></td>
<td>F-actin capping protein a</td>
<td>P28495</td>
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<td>0</td>
<td>0.014</td>
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<td>Cofilin 1</td>
<td>P23528</td>
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<td>Filamin-A</td>
<td>P21333</td>
<td>9</td>
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<td>0.003</td>
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<td></td>
<td>Moesin</td>
<td>P26038</td>
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<td>0</td>
<td>0.001</td>
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<tr>
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<td>Myosin heavy chain</td>
<td>P35579</td>
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<td>1</td>
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<tr>
<td></td>
<td>Talin 1</td>
<td>Q9Y490</td>
<td>8</td>
<td>1</td>
<td>0.0002</td>
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<td></td>
<td>Thb4</td>
<td>P62328</td>
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<td>3</td>
<td>0.0001</td>
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<td></td>
<td>Tubulin α 1</td>
<td>P68366</td>
<td>2</td>
<td>0</td>
<td>0.002</td>
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<td>VASP</td>
<td>P50552</td>
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<td>Zyxin</td>
<td>Q15942</td>
<td>4</td>
<td>0</td>
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</table>

* Rounded to whole numbers.

b VASP, vasodilator-stimulated phosphoprotein.
concentrations of cytoskeletal proteins and their fragments and that both of these phenomena reflect key processes of psoriatic pathogenesis.

Calgranulins A and B (S100A8 and S100A9, respectively) are Ca²⁺-binding proteins that are produced in neutrophils, monocytes, and epithelial cells and are secreted from cells as the A/B heterodimer (known as calprotectin) (28). Calgranulins are known to play a role in a genetic predisposition to psoriasis and in the differentiation of keratinocytes (29). Ca²⁺ homeostasis of the epidermis, and potentially calgranulins, is involved in the formation of the skin barrier, which is known to be impaired in psoriasis, and in cytokine activation of lymphocytes. In addition, the calgranulin genes are overexpressed in the psoriatic epidermis with respect to both mRNA and protein concentrations, and the calprotectin concentration in serum has been correlated with disease activity (14). All of these findings point to a potential, although so far unproved, role for calgranulins in psoriasis pathogenesis.

Calgranulins A and B have also been demonstrated to colocalize with cytoskeletal structures in activated monocytes in a Ca²⁺-dependent manner and to act as messengers regulating cytoskeletal changes (30). Importantly, marked alterations in the cytoskeleton/extracellular matrix protein-connection system are observed in psoriatic skin. These observations suggest a link between increased calgranulin concentrations and the increased concentrations of cytoskeletal proteins and peptides we observed in this study. The observed role of Ca²⁺ homeostasis in mediating Ca²⁺-dependent proteolysis of cytoskeletal proteins strengthens this hypothesis and implicates Ca²⁺-dependent proteases, such as caspases or calpains, in the process. We propose a mechanism in which calgranulin overproduction leads to the disturbance of Ca²⁺ homeostasis in the epidermis and to altered intracellular and extracellular relocalization of cytoskeletal components, which changes the presentation of these components upon cell damage or death. In addition, disturbances in Ca²⁺ concentrations within and outside cells may trigger abnormal proteolysis of cytoskeletal components, leading to increases in the concentrations of cytoskeletal pro-

Fig. 1. Relative concentrations of actin γ, talin 1, and their proteolytic peptides in the plasma of psoriasis patients and healthy control individuals.

Data are presented as the median and range.

Fig. 2. Relative concentrations of selected proteolytic peptides from Thb4 (A) and talin 1 (B) in plasma samples from psoriasis patients and healthy control individuals.

Thb4 peptide 1, AcSDKPDMAEIEKFDSKLK; Thb4 peptide 2, E.TQEKNPLPSKTEIEKQAGES; Thb4 peptide 3, E.KNPLPSKETIEKQAGES; talin 1 peptide 1, A.SNPEFSSIPAQISPEGR.A; talin 1 peptide 2, S.PEPPAKTSTPEDFIR.M. Data are presented as the median and range. The 2 periods in the peptide amino acid sequences indicate the beginning and end of the identified peptide. Letters before and after a period indicate the amino acid residues immediately preceding and following the identified peptides, respectively. The absence of a period at one of the ends indicates the N or C terminus of the protein. AC, acetylated N-terminal amino acid residue.
tein–derived peptides in the circulation. Another hypothesis is that altered presentation or increased concentrations of circulating cytoskeletal proteins and peptides may lead to an immune response against them.

Cytoskeletal proteins play a vital role in a variety of biological processes, such as cell–cell and cell–matrix adhesion, cell proliferation, and cell migration, that are important in the etiology of psoriasis. The cytoskeleton also regulates the organization of immunologic syn-

Fig. 3. Thb4, calgranulin, and calprotectin concentrations in plasma samples from psoriasis patients and healthy control individuals.

(A), Thb4 concentrations in plasma of psoriasis patients and healthy controls as assessed by an ELISA specific for the N terminus of Thb4 (amino acid residues 1–14) and by an ELISA developed in-house that is specific for full-length Thb4 (residues 1–43).

(B), Relative concentrations of calgranulin A and calgranulin B (spectral counts) and concentrations of calprotectin (ELISA measurements). Data are presented as the median and range in both panels.

Fig. 4. Numbers and relative concentrations of proteolytic peptides from fibrinogen chains Aα and Bβ in plasma samples from psoriasis patients and healthy control individuals.

(A), Numbers of proteolytic peptides from fibrinogens Aα and Bβ. (B), Relative concentrations of selected proteolytic peptides from fibrinogen chains Aα and Bβ. Peptides A.DS.GEGDFLAEGGGVRG, K.S.S.YSKQFSTSTSYNRGDS.F.ESSKSY.K, and P.GST.GRN.PGS.GTGTATW.KP.GSSGP.G are from the fibrinogen Aα chain; peptide R.EEAPSLRP.A.PPISG.GGY.R. is from the fibrinogen Bβ chain. Data are presented as the median and range in both panels. The 2 periods in the peptide amino acid sequences indicate the beginning and end of the identified peptide. Letters before and after a period indicate the amino acid residues immediately preceding and following the identified peptides, respectively. The absence of a period at one of the ends indicates the N or C terminus of the protein. Ac, acetylated N-terminal amino acid residue.
apases and cellular activation via interaction of focal adhesion proteins with the cytoplasmic tails of integrins. Focal adhesion proteins include talin, vinculin, paxillin, filamin, and vasodilator-stimulated phosphoprotein (VASP), and several of these proteins are increased in psoriatic plasma. Talin 1 has recently emerged as a crucial regulator of integrin functions in numerous cell types. Proteolysis of talin 1 by calpain has been proposed to regulate adhesion complexes during cell migration. It is possible that changes in calpain-mediated proteolysis of talin 1 are related to the disturbance of Ca\textsuperscript{2+} homeostasis discussed above and lead to increased concentrations of talin peptides and other components of focal adhesions in the circulation.

One of the proteins from the cytoskeletal group that we investigated in greater detail was Thb4, which exhibited the highest increases in concentrations of proteolytic peptides in psoriatic plasma. These data, along with the ELISA data that showed only a moderate increase in the concentration of the intact Thb4 molecule, suggest that the observed increases in the C-terminal peptides are largely due to increased proteolytic activity toward Thb4 and less to an increase in the concentration of the intact protein. This conclusion also implies that C-terminal Thb4 peptides exhibit a conformation that is not readily recognized by the Thb4-specific antibody we used. The absence of commercially available antibodies with specificities that would confirm apparent increases in C-terminal peptides emphasizes the general concern that because of the high specificities of antibodies, ELISAs may not always be suitable for validation of discovered biomarkers. The use of mass spectrometry–based methods for validating discovered protein and peptide biomarkers may be a more suitable approach in some cases.

The presence of Thb4 fragments in nonpathologic serum has previously been reported; however, the number of peptides identified in nonpathologic plasma has been shown to be substantially lower than in serum (10, 24). Such results are in agreement with our observation of only a small number of Thb4 peptides in control plasma. This finding is most probably attributable to the absence of the activation of the coagulation cascade in plasma (10, 24), suggesting that plasma samples may be preferred for peptidomic analyses. Thb4 is a small peptide (4.9 kDa) found in the circulation and in a variety of tissues and cell types, with the highest concentrations occurring in platelets and polymorphonuclear leukocytes (31). The molecule serves as an actin-sequestering peptide, but it also plays important roles in cell proliferation, migration, differentiation, induction of metalloproteases, and angiogenesis (32–34). High Thb4 concentrations have been observed in wound fluid (35), suggesting the peptide’s importance in wound healing, a process that has numerous similarities to the hyperproliferative and angiogenic state of psoriatic skin. Furthermore, the proposed Thb4 property of binding to multiple ligands suggests that it may have an integrative function that links the actin cytoskeleton to important immune and cell growth signaling cascades (36).

Thb4 also plays an important role in cytoskeleton reorganization and has been reported to up-regulate the production and translocation to the nucleus of zyxin, another cytoskeletal protein (37) whose fragments are increased in psoriatic plasma. In addition, tissue and plasma transglutaminases were recently suggested to be able to incorporate Thb4 into fibrinogen and fibrin molecules (38, 39). Huff et al. suggested that such binding provides a mechanism for increasing the local Thb4 concentration near sites of clotting and tissue damage (39). Intriguingly, the binding sites of Thb4 (38) are all within the portion of the molecule identified to be highly increased in psoriatic plasma (see Fig. 4 in the online Data Supplement). It is therefore possible that Thb4 binding to fibrinogen is one reason for the detection of highly increased concentrations of C-terminal fragments of Thb4 in plasma. The cleaved N-terminal portion of the molecule may be rapidly cleared from the circulation, whereas C-terminal fragments may bind to fibrinogen or large fibrinogen peptides, remain in the circulation much longer, and thereby accumulate. Fibrinogen may function as a molecular sponge and carrier for Thb4 C-terminal fragments, similarly to the function, proposed by Liotta and Petricoin, of albumin as a carrier of LMW proteins and peptides in the circulation (11, 12).

We also observed decreased fibrinogen proteolysis, which was manifested as decreased numbers and concentrations of its fragments in psoriatic plasma compared with controls. Some of the fibrinogen peptides with decreased concentrations (see Fig. 2 in the online Data Supplement) have also been shown by Villanueva et al. to be significantly decreased in the sera of thyroid cancer patients (22). These similarities suggest that an alteration in fibrinogen proteolysis reflects common mechanisms inherent to autoimmunity and inflammation. In addition, the detected changes in fibrinogen proteolysis may be relevant to the increased risk of cardiovascular diseases in psoriatic patients. Finally, given that certain posttranslational modifications of fibrinogen, such as citrullination, have been shown to predict the onset and future severity of rheumatoid arthritis (40), altered proteolysis of fibrinogen due to changes in its posttranslational modifications may represent a link between psoriasis and future development of psoriatic arthritis.

This study has demonstrated that proteomic and peptidomic methods that do not require extensive
sample preparation and fractionation can be useful in identifying candidate biomarkers present in human plasma. A combined proteomic and peptidomic evaluation of plasma may provide more detailed information on the candidate markers and the processes that lead to their appearance in the circulation.

The results presented in this study require further validation and may currently be viewed as hypothesis-generating and spurring further research. The next steps are to determine the value of the identified proteins and peptides in the relevant areas of psoriasis research. Identified markers may be evaluated in longitudinal studies of either a prospective or retrospective nature. We plan to follow up psoriasis patients for longer periods to correlate the identified markers with disease activity, the risk of developing a severe form of the disease, or the risk of developing psoriatic arthritis in the future. The targeted approach, such as ELISA and multiple reactions monitoring with mass spectrometry, will be used in these studies.

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References


