

Increased circulating anti- $\alpha 6$  integrin autoantibodies in psoriasis and psoriatic arthritis  
but not in rheumatoid arthritis

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**Short running title:** Anti- $\alpha 6$  integrin antibodies in psoriasis

## ABSTRACT

In the psoriatic skin laminin integrity is altered, which could lead to insufficient laminin integrin interactions, leaving the  $\alpha 6$ -integrin exposed and possibly accessible for autoantibody production. Therefore we investigated the presence of anti- $\alpha 6$  integrin autoantibodies in the serum of patients with psoriasis vulgaris (Ps), psoriatic arthritis (PsA) and rheumatoid arthritis (RA) in comparison to healthy donors. The level of circulating anti- $\alpha 6$  integrin antibodies was determined by ELISA using  $\alpha 6$  integrin fragments. Antibodies against at least one recombinant fragment were found in approximately 30% of Ps and PsA patients. In contrast, in RA patients the frequency of antibodies was similar to healthy controls. Our study shows the presence of anti- $\alpha 6$  integrin antibodies in Ps and PsA but not in RA, which could indicate ongoing abnormal processes in the skin. Anti- $\alpha 6$  integrin autoantibodies may contribute to the formation of micro-wounds in the skin and to the characteristic wound healing phenotype in psoriasis.

alpha-6 integrin      antibodies      psoriasis      psoriatic arthritis      rheumatoid  
arthritis

## INTRODUCTION

Psoriasis is a chronic, immune-mediated disease with predominantly skin and/or joint manifestation affecting approximately 1.5-2% of the population worldwide. The disease is not homogenous in its clinical presentation, but the most frequent form of plaque type psoriasis vulgaris (Ps) is characterized by chronic inflammation of the skin resulting in scaly, raised, well-demarcated erythematous plaques. In 20% of the cases psoriasis is associated with arthritis or other types of musculoskeletal involvement such as enthesitis, spondylitis or dactylitis, causing severe pain, morning stiffness, soft-tissue swelling and occasionally leading to the deformation of joints <sup>1</sup>. Psoriatic arthritis (PsA) has several features in common with psoriasis, but it is also considered a distinct disease <sup>2</sup>. PsA has some similarities radiographically to rheumatoid arthritis (RA), and occasionally it may be difficult to differentiate between the two joint diseases especially in patients with mild cutaneous signs <sup>3</sup>.

The pathogenesis of psoriasis is not yet fully understood. It is considered a multifactorial genetic inflammatory disease, triggered by environmental factors. Recent studies have suggested that T helper 1, T helper 17 lymphocytes and their characteristic cytokines are primary modulators of inflammation in psoriasis <sup>4,5</sup>. Data indicate that keratinocyte hyperproliferation triggered by skin-infiltrating T cells plays an important role in the formation of psoriatic lesions <sup>6</sup>. A few reports have also demonstrated autoantibody production including antinuclear, anti-double-stranded DNA and anti-thyroid microsomal antibodies <sup>7-10</sup>.

Integrins are known to mediate cell-cell and cell-extracellular matrix interactions and signaling influencing cell survival, differentiation, migration and apoptosis <sup>11,12</sup>.

There are 24 different members of integrins, among them the  $\alpha6\beta4$  integrin plays an important role in cell to extracellular matrix connections. Together with  $\alpha6\beta1$ , the  $\alpha6\beta4$  integrin is the major laminin-binding receptor on the cell surface<sup>13</sup>. Laminin can be detected around the synovial lining cells in normal and in less inflamed rheumatoid synovium, but in RA with severe inflammation laminin expression is diminished<sup>14</sup>. The  $\alpha6\beta4$  integrin is expressed by epidermal keratinocytes and localized primarily at the basal surface of basal cells, where it plays a fundamental structural role in the formation of hemidesmosomes<sup>15</sup>. In the healthy skin, basal keratinocytes are tightly and continuously anchored to the basement membrane via their integrin receptors at the dermal-epidermal junction (DEJ). The  $\alpha6\beta1$  integrin is strongly expressed in the normal synovial tissue by synovial lining cells<sup>14</sup>. It is known that there is no basement membrane in the normal synovium, but the presence of laminin has been shown in the extracellular matrix around synovial cells. On the other hand, in RA with a severe inflammation, both laminin as well as the  $\alpha6$  integrin subunit expression is absent. The complete lack of the ligand may explain the downregulation of  $\alpha6$  integrin<sup>16</sup>.

The  $\alpha6$  integrin knockout mice died shortly after birth with severe blistering defects of the skin<sup>17</sup>. *Niculescu C.* et al. had investigated the effect of the loss of the endothelial  $\alpha6$  integrin subunit using  $\alpha6^{fl/-}$ -K14-Cre-ER<sup>T2</sup> mice. They found that the mutant epidermis resembled many histological features of inflammatory skin disorders, such as psoriasis, with epidermal hyperplasia, hyperkeratosis and infiltration of immune cells<sup>18</sup>.

Because alteration in laminin integrity is described in psoriatic uninvolved and involved skin<sup>19,20</sup>, we hypothesized, that this could lead to insufficient laminin integrin interactions leaving the integrin exposed and accessible to immune cells, which may trigger the production of anti  $\alpha6$ -integrin autoantibodies.

Thus, the aim of this study was to investigate the presence of circulating anti- $\alpha 6$  integrin autoantibodies in the serum of patients with Ps and PsA.

## MATERIALS AND METHODS

### PATIENTS

We used serum samples from 62 patients with Ps, 46 patients with PsA, 52 healthy blood donors and, as an additional control group, 54 RA patients to investigate for the presence of anti- $\alpha 6$  integrin autoantibodies. The study was approved by the Human Investigation Review Board of the University of Szeged and carried out in compliance with the Helsinki declaration. Serum samples were collected at regular visits, regardless of ongoing treatments or clinical status of the patients. All psoriatic patients displayed skin lesions and the PsA was clinically and radiologically verified. Demographic and clinical data of the patients are presented in Table 1.

### ANTIGENIC EPITOPES WITHIN THE HUMAN $\alpha 6$ INTEGRIN PROTEIN

The sequence of human  $\alpha 6$  integrin from the Swiss Prot and TrEMBL databanks was analyzed with the Wisconsin Package, Version 8 software (Genetic Computer Group, Madison, WI) using the PeptideStructure and PlotStructure functions to select three fragments (abbreviated as TPAC, SVLP, SPDA) predicted to have elevated immunogenic properties <sup>21</sup>. Bovine serum albumin (BSA) conjugated dimers of immunogenic peptide constructs were obtained from ProteoGenix.

## DETECTION OF ANTI- $\alpha$ 6 INTEGRIN ANTIBODY BY ELISA

Antigenic peptide constructs were dissolved and diluted in phosphate-buffered saline (PBS, pH 7.4). Microplates were coated overnight with antigen solutions of 10  $\mu$ g/ml concentration at 4 °C. After washing the wells with distilled water blocking was performed with PBS containing 5 % of FBS for 1.5 hours at 37 °C. Serum samples of patients and healthy controls were diluted 1:200 in PBS-Tween (PBST), and incubated in the wells for 1 hour at 37 °C followed by extensive washing with PBST. Biotinylated anti-human IgG antibody (1  $\mu$ g/ml, Vector Laboratories) was added to each well and plates were incubated for 1 hour at 37 °C. After washing with PBST plates were incubated with ExtrAvidin™-Peroxidase (Sigma-Aldrich, USA) for 30 minutes at 37 °C. Plates were extensively washed with PBST and 100  $\mu$ l substrate solution (17 mg o-phenylenediamine dissolved in 50 ml phosphate-citric acid buffer, pH 5.0, containing 20  $\mu$ l 30% H<sub>2</sub>O<sub>2</sub>) was added to each well. The color reaction was allowed to develop for 5-10 min at room temperature in the dark. The reaction was stopped by the addition of 4N H<sub>2</sub>SO<sub>4</sub> and optical density (OD) was measured at 492 nm.

Cut-off values were calculated by  $MV \pm 2SD$  (MV: mean OD value of healthy control subjects, SD: standard deviation of the control ODs). Ten healthy samples representing the cut-off value of the 52 healthy controls were used of all measurements. Patients displaying autoantibodies against at least one antigenic epitope of  $\alpha$ 6 integrin were considered as positive.

## STATISTICS

Statistical analysis was performed with two way chi-square test followed by Holm-Bonferroni correction.

## RESULTS

Ps patients showed a positive reaction with a higher frequency against the antigenic epitopes (11.3% for TPAC and 21% for SVLP), compared to PsA patients (8.7% for TPAC and 17.4% for SVLP). However, autoantibodies for the SPDA antigenic peptide were detected more often in the PsA sera (Ps 3.2% and PsA 8.7%). Although the observed differences did not reach the level of statistical significance, a strong tendency towards a higher level of autoantibodies recognizing TPAC and SVLP epitopes were observed more frequently in patients with Ps and PsA, compared to healthy controls (3.8% for TPAC, 5.8% for SVLP, 5.8% for SPDA). In contrast, patients with RA displayed antibodies in similar proportion as those of the healthy control group (3.7% for TPAC, 5.6% for SVLP, 3.7% for SPDA).

Next we examined the distribution of antibodies against at least one recombinant epitope of  $\alpha 6$  integrin in patients with Ps and PsA, RA and healthy controls. Antibodies against at least one recombinant epitope of  $\alpha 6$  integrin were found in 30.65% and 28.26% of patients with Ps and PsA, respectively. Whereas, anti- $\alpha 6$  integrin antibodies were only present in 9.3 % of the patients with RA (Figure 1.) For the comparison of patients with Ps, PsA, RA and healthy controls we used the two way chi-square test. The test resulted in a p-value of 0.0154, indicating that differences between the different groups did not occur by chance. 10.5% of positive Ps and 23.1% of positive PsA patients presented autoantibodies to more than one antigenic peptides of  $\alpha 6$  integrin. One Ps patient and one RA patient displayed autoantibodies against all of the three chosen antigenic peptides.



Positivity for anti- $\alpha 6$  integrin antibodies displayed an inverse significant correlation to the age of onset of psoriasis. Approximately 36.7% of patients who exhibited early appearance of disease were positive for anti- $\alpha 6$  integrin antibodies, while patients with late disease onset presented autoantibodies only in 7.7%.

About 71% of the Ps patients received some form of systemic treatment at the time of blood sampling and almost all of the patients with PsA were treated with systemic therapy (96%). In patients who received systemic therapy, the occurrence of anti- $\alpha 6$  integrin antibodies was detected to a similar extent (31.8%) as in the non-systemic therapy group (27.8%).

## DISCUSSION

The altered activation of the cellular immune system in psoriasis has been in focus of research for decades. In contrast, the role of the humoral immune response in the pathogenesis of psoriasis has only been recognized recently, and relatively few data are available in the literature regarding circulating autoantibodies. Recent studies have shown the presence of antibodies against the protease inhibitor calpastatin, anti-stratum corneum antibodies, anti-thyroid microsomal antibodies, antinuclear antibodies and anti-double-stranded DNA antibodies in Ps patients, and concluded that latent autoimmune disease may develop in Ps patients, without any clinical symptoms resulting from the formation of these antibodies<sup>7-9,22,23</sup>. The presence of the anti-cyclic citrullinated peptide antibody has also been demonstrated in patients with Ps and PsA<sup>10,24</sup>. However, no specific autoantibody was identified to be characteristic of all patients with psoriasis. It is also unclear whether the autoantibodies present in psoriasis are involved in the pathogenesis of psoriasis.

Previous studies using immunostaining and confocal laser scanning microscopy demonstrated structural alterations of the basement membrane characterized by irregular  $\gamma 1$  subunit of laminin integration in involved and uninvolved psoriatic skin<sup>19,20</sup>. Another study has reported irregular distribution of  $\alpha 2$  laminin chain at the DEJ in involved psoriatic lesions<sup>25</sup>. Further studies have demonstrated that the perturbation of basement membrane was one of the very earliest changes during the development of psoriatic lesions. This finding suggests a potential antigen localized at the DEJ playing a role in the initiation of the disease. In addition, it has been shown that the disruption of basement membrane is specific for psoriasis compared to other common inflammatory diseases<sup>26</sup>.

We hypothesized that alteration in laminin integrity in the psoriatic skin leads to insufficient laminin integrin interactions, thereby leaving the integrin exposed and accessible to immune cells, which may trigger the production of anti  $\alpha 6$ -integrin autoantibodies. The autoantibodies could only indicate ongoing abnormal processes in the skin without taking part in the pathomechanism, but they could also contribute to the formation of micro-wounds in the skin, therefore participating in the induction of a chronic wound healing phenotype, characteristic of the psoriatic skin.

The presence of circulating anti- $\alpha 6$  integrin antibodies were also shown in other autoimmune diseases like oral pemphigoid and bullous pemphigoid where altered integrin mediated interactions may play a role in blister formation<sup>21,27</sup>. The coexistence of psoriasis and bullous pemphigoid was revealed by *Grattan* in 1985<sup>28</sup>. Over time, several studies have reported on the relationship of both diseases. A rare autoimmune blistering disease, anti-p200 pemphigoid, which is characterized by antibodies against the  $\gamma 1$  laminin chain, shows co-association with psoriasis in approximately 30% of patients. *Ohata* et al. reported bullous pemphigoid as the most common comorbidity in the population of patients with coexisting psoriasis and autoimmune blistering disease, followed the anti-p200 pemphigoid<sup>29</sup>.

Previous studies stated as the common feature in bullous pemphigoid and psoriasis to be the damaged basement membrane. Structural changes of BM proteins may generate altered antigenicity, thereby leading to formations of various anti-basement membrane zone antibodies. Additionally, occurrence of several nonpathogenic autoantibodies may follow from „epitope spreading”<sup>30,31</sup>.

*Kobayashi* et al. showed blisters limited exclusively to the psoriatic plaques. They suggested that due to enzymatic degradation of the basement membrane zone antigens could unmask in psoriatic lesions, and become available to circulating antibodies<sup>31,32</sup>.

In the current study, we found positivity for anti- $\alpha 6$  integrin autoantibodies approximately 31% and 28% of patients with Ps and PsA, respectively. It is worth noting that all patients with PsA also had skin lesions. RA patients served as a control group to clarify the connection between the occurrence of these autoantibodies and joint involvement. Among RA patients 9.3%, while in healthy controls 15.4% displayed antibodies. The combined analysis of all four groups (Ps, PsA, RA, healthy) for  $\alpha 6$  integrin positivity using the two way chi-square test indicated that differences among the groups did not occur by chance. However, when the groups were compared two by two differences did not reach the level of statistical significance.

In our Ps and PsA patients skin symptoms were present, in contrast, RA only affects the joints, indicating that that the primary antigen source of anti- $\alpha 6$  integrin antibodies is likely to be located in the skin. Although our healthy control group included volunteers with no manifest psoriasis, PsA or RA, we can not rule out the possibility that increased anti- $\alpha 6$  integrin antibodies could indicate susceptibility to develop Ps or PsA. It is known that in systemic lupus erythematosus the development of autoantibodies may precede, even by years, the appearance of clinical manifestations<sup>33</sup>. Patients undergoing systemic treatments displayed autoantibodies at the same extent as patients who did not receive systemic therapy in our study, indicating that therapies had no impact on anti- $\alpha 6$  integrin antibody production.

Two forms of psoriasis can be distinguished based on the onset of the disease. Type 1 psoriasis shows early onset ( $\leq 40$ years), and has a strong genetic component

(association with HLA-Cw6). Type 2 psoriasis manifests after 40 years of age and family members are usually not affected<sup>34,35</sup>. In patients with early onset Ps the positivity for anti- $\alpha$ 6 integrin antibodies was significantly more frequent than in those of late onset (p=0.0435), however, in the serum of PsA patients this correlation was not present.

This observation is well in line with the study of *Dalmády* et al. where similar inverse correlation between the level of anti-mutated citrullinated vimentin and the age of Ps onset was detected, while this correlation was not seen in the PsA group<sup>24</sup>.

Our study provides evidence for the presence of anti- $\alpha$ 6 integrin antibodies in psoriasis. Whether the observed autoantibody production is exclusively a result of the pathomechanism or it excites the development of the disease still remains unclear, and requires further elucidation.

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CONFLICT OF INTEREST: None declared



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**FIGURE LEGENDS:**

Figure 1. Distribution of the presence of all the three investigated autoantibodies in the different groups. The presence of at least one antigenic epitope of  $\alpha 6$  integrin was considered as positive for anti- $\alpha 6$  integrin autoantibodies. Columns diagram of bar chart shows the number and below the percentages of positive cases are indicated. The total numbers of investigated serum samples are shown in brackets.

Table I. Basic demographic and clinical characteristics of psoriasis vulgaris and psoriatic arthritis patients groups. MTX: methotrexate; Biological therapy: TNF inhibitors (infliximab, adalimumab, etanercept, golimumab), IL 12/23 inhibitor; PUVA: psoralen + ultraviolet A; 311 nm NB-UVB: 311 nanometer narrow-band ultraviolet B.

<b>Variable</b>	<b>Psoriasis Vulgaris (n=62)</b>	<b>Psoriatic Arthritis (n=46)</b>
Male/Female ratio	41/21	26/20
Age (years) mean±SD	47.5±13.44	50.75±10.65
<b>Therapy</b>		
MTX or leflunomide therapy	33/62	31/46
Biological therapy	4/62	28/46
Retinoid	3/62	1/46
Cyclosporin A	1/62	0/46
PUVA/311 nm NB-UVB	3/62	1/46
Only local steroid	7/62	2/46
Not treated	11/62	0/46

