Humoral Autoimmune Responses to the Squamous Cell Carcinoma Antigen Protein Family in Psoriasis

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Substantial evidence indicates that psoriasis is a T-lymphocyte-mediated autoimmune disease. However, longstanding data also indicate IgG and complement deposition in upper epidermis of psoriasis plaques. This led us to propose that autoantigen–autoantibody interactions in the skin may also be of pathogenic importance. Here, we have confirmed the presence of IgG in upper lesional epidermis and used high-resolution two-dimensional immunoblotting of extracts from this tissue, and laser desorption mass spectrometry of tryptic peptides, to define a series of epidermal proteins that bind IgG from psoriatic serum. The most prominent of these autoantigens are homologues of the serpin, squamous cell carcinoma antigen (SCCA), the other autoantigens identified including arginase 1, enolase 1, and keratin 10. Blood levels of IgG autoantibodies that bind to SCCA proteins were significantly higher in psoriasis than healthy controls ($P=0.005$), but were not detectable in sera from patients with active atopic dermatitis. To our knowledge, SCCA proteins have not previously been described as autoantigenic in animals or humans and form complexes with IgG that are associated with complement deposition. These findings expose potentially pathogenic humoral immunologic events and thus possible therapeutic targets in psoriasis.

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INTRODUCTION

The skin lesions or plaques of psoriasis are characterized by epidermal hyperplasia and infiltrates of leukocytes including neutrophils and homing receptor-positive T lymphocytes (Gudjonsson *et al.*, 2004). The disease can be provoked by activated T cells in xenograft models (Boyman *et al.*, 2004) and therapies that target T cells are effective (Kupper, 2003), although potentially toxic in the long term. Analysis of T-cell receptor diversity indicates oligoclonal infiltrates in many, if not all psoriatic lesions, and thus an antigen-driven process (Menssen *et al.*, 1995; Vekony *et al.*, 1997). These and other findings, including association with HLA-Cw*0602 and inflammatory arthropathy, indicate that psoriasis is an autoimmune disease that occurs in genetically predisposed individuals (Gudjonsson *et al.*, 2004). Although these findings show that T-cell-mediated events are of key pathogenic importance, it was reported more than 30 years ago that there is more IgG bound *in vivo* in the upper epidermis and stratum corneum of psoriatic lesions than in a variety of other inflammatory and hyperkeratotic skin diseases (Jablonska *et al.*, 1975). Reports also describe the presence of products of complement activation in the surface stratum corneum of plaques, including C5a/C5a des arg (Schröder and Christophers, 1986) and C5-9/membrane attack complex (Takematsu and Tagami, 1992). These data suggest that antigen–antibody interactions, which provoke complement activation, are of pathogenic relevance in psoriasis.

A previous attempt to identify autoantigens in psoriatic lesions by screening cDNA expression libraries with psoriatic serum defined three putative autoantigens, including heterogeneous nuclear ribonucleoprotein-A, keratin 13 (K13), and a previously uncharacterized protein (Jones *et al.*, 2004). Assay of IgG titers was complicated by phage reactivity requiring extensive pre-clearing of serum, and these titers were no different in psoriatic and control sera. Apart from this, there have been no reports of the systematic analysis of psoriatic lesional proteins that bind IgG. The rapidly shed and readily harvested surface stratum corneum of psoriatic lesions is a useful source of biologically active molecules that occur in the underlying viable epidermis. Analyses of extracts of this material have previously allowed demonstrations of the *in vivo* production of leukotriene B_4 (Brain *et al.*, 1984), IL-8 (Schröder and Christophers, 1986; Fincham *et al.*, 1988; Gearing *et al.*, 1990), tumor necrosis factor-α (Ettehadi *et al.*, 1994), and β-defensins (Harder *et al.*, 1997, 2001). We have therefore used stratum corneum as a source of lesional proteins to define IgG-reactive autoantigens in psoriasis.

RESULTS

IgG deposition in the upper epidermis of psoriasis lesions

Lesional stratum corneum was obtained from an informed, consenting volunteer with extensive, untreated chronic

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Abbreviations: Ab, antibody; AP, alkaline phosphatase; K, keratin; PBS, phosphate-buffered saline; PBS/T, PBS containing 0.05% Tween 20; PBS/T/M, PBS/T containing 1% skimmed milk powder; SCCA, squamous cell carcinoma antigen

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plaque psoriasis, and aqueous extracts were prepared as described in Materials and Methods. Total proteins in supernatants of these extracts were used to coat microtiter plate wells, and the presence of IgG was demonstrated by the use of alkaline phosphatase (AP)-labeled goat anti-human IgG either in the absence of added serum or after the addition of 100-fold diluted, pooled psoriatic serum (Figure 1). IgG was shown in stratum corneum extracts, and the amount of detected IgG could not be enhanced by the prior addition of psoriatic serum, which suggested that target molecules were substantially bound by IgG *in vivo*. This supports the longstanding findings of Jablonska *et al.* (1975), who reported IgG deposition in upper lesional epidermis by the use of direct immunofluorescence, which could not be usually enhanced by prior incubation of sections with psoriatic serum.

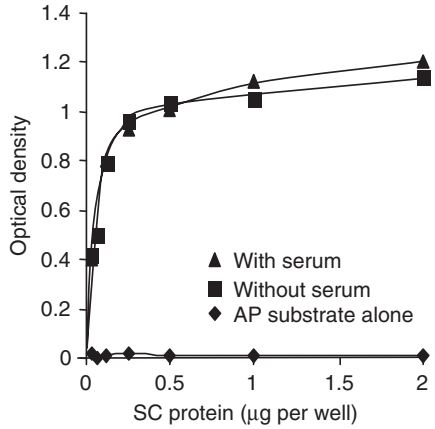

Figure 1. IgG is present in the stratum corneum (SC) of psoriasis lesions. The ELISA used AP-labeled Ab to detect IgG in aqueous stratum corneum extract, with and without prior addition of pooled, 100-fold diluted serum from eight psoriasis patients. The lowest curve shows optical readings in the presence of AP substrate alone, without added AP-labeled Ab. This indicates that there is no significant endogenous AP activity in the stratum corneum samples. Each point represents the mean of quadruplicate estimations.

Identification of IgG-reactive proteins in the stratum corneum of psoriasis lesions

Protein extracts were prepared by homogenization of psoriatic lesional stratum corneum and further solubilized by the addition of urea, thiourea, and non-ionic detergent. In these extracts, constituent proteins were successively separated by high-resolution isoelectric focusing and denaturing PAGE. Two-dimensional gels were prepared in the same electrophoresis tank and were either stained with colloidal Coomassie blue (Candiano *et al.*, 2004) (Figure 2a) or electroblotted onto nitrocellulose and incubated with pooled psoriatic serum (1:200) (Figure 2b) or pooled serum from age- and sex-matched healthy controls (Figure 2c). Bound IgG, detected with AP-labeled anti-human IgG, showed the presence of a series of IgG-reactive proteins that were more numerous on blots incubated with psoriatic sera than with control sera. Spots labeled 1–11 (Figure 2b and c) corresponded with similarly numbered proteins on the Coomassie-stained gel (Figure 2a). Appropriate Coomassie-stained protein spots were cut from the gel, subjected to trypsin digestion, and tryptic peptides analyzed by MALDI-TOF (matrix-assisted, laser desorption ionization-time of flight) mass spectrometry (Shevchenko *et al.*, 1996; Bonetto *et al.*, 1997). The proteins identified included enolase 1, squamous cell carcinoma antigen (SCCA), SCCA 1, SCCA 2, SCCA 2b, arginase 1, and K10, as indicated in the lower panel of Figure 2.

Quantification of SCCA- and K10-reactive IgG in blood samples from psoriasis patients and controls

In repeated two-dimensional immunoblots of separated stratum corneum proteins, incubated with sera from

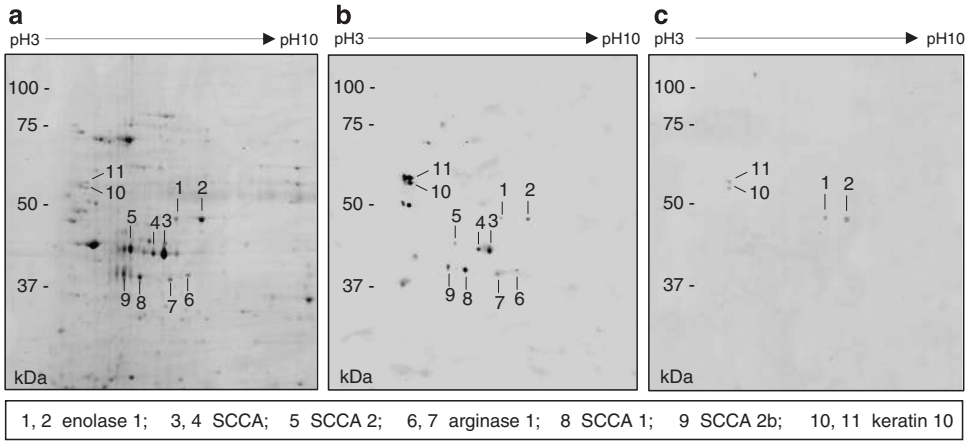

Figure 2. IgGs in pooled serum from psoriasis patients recognize a greater range of proteins in lesional stratum corneum extract than pooled serum from age- and sex-matched controls. (**a**) Coomassie-stained two-dimensional gel showing separated proteins. Blots of 2-dimensional gels run in parallel were incubated with pooled serum (1:200 dilution) from eight patients with psoriasis (**b**) and eight age- and sex-matched healthy controls (**c**), and IgG-binding proteins localized with AP-labeled anti-IgG. IgG-reactive proteins that corresponded with Coomassie-stained spots in panel a were identified by MALDI-TOF mass spectrometry and are labeled 1–11. The identities of these proteins are indicated in the lower panel. Similar results to those shown in panels a–c were obtained in a second experiment with different stratum corneum extract and pooled serum samples.