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Non-constitutive epidermal antigens that cross-react with streptococcal antigens in psoriasis.

Original Article

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SUMMARY.

Introduction. It was previously demonstrated by immunohistochemical analysis that there are antigens in psoriatic lesional skin that are recognized by autoantibodies in psoriatic patients and mouse antibodies to streptococcal antigens. We looked for healthy-skin-derived keratinocyte antigens recognized by those antibodies to determine whether those skin antigens are constitutive or are expressed during the development of the epidermal lesions.

Material and Methods. An extract of keratinocytes was prepared from six healthy skin biopsies. 96 microwell plates were sensitized with the keratinocyte extract to test the antibody recognition by sera from 26 psoriatic patients and 26 healthy donors, and two mice policlonal sera against total soluble extracts from *S. pyogenes*, with and without heat shock induction at 42°C.

Results. Healthy skin keratinocyte extract was recognized by both anti-streptococcal antisera. No significant differences were found between them. Sera from psoriatic patients did not show recognition of the antigens present in the extract of keratinocytes.

Conclusions. It seems likely that there are constitutive epidermal antigens that cross-react with *S. pyogenes*. Although the presence of autoantibodies that cross-react with streptococcal antigens has been reported, our results suggest that the target of those autoantibodies could be expressed during the development of disease because they are not present in the healthy skin.

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Key words: Psoriasis, Autoimmunity, Autoantigens, *Streptococcus pyogenes*.

RESUMEN.

Antígenos epidérmicos no constitutivos con reactividad cruzada hacia antígenos estreptocócicos en la psoriasis.

Introducción. Se ha demostrado por análisis inmunohistoquímico que en las lesiones psoriásicas hay

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antígenos que son reconocidos por autoanticuerpos en el suero de los pacientes psoriásicos y por anticuerpos de ratón contra antígenos estreptocócicos. En este trabajo realizamos la búsqueda de antígenos de queratinocitos de piel humana sana que son reconocidos por esos anticuerpos con la finalidad de determinar si se trata de antígenos constitutivos o éstos se expresan durante el desarrollo de las lesiones epidérmicas.

Material y métodos. Se preparó un extracto de queratinocitos a partir de seis biopsias de piel sana. Se sensibilizaron placas de 96 pozos con el extracto de queratinocitos para probar el reconocimiento por el suero de 26 pacientes psoriásicos y 26 donadores sanos, así como dos sueros policlonales de ratón contra extractos solubles totales de *S. pyogenes* obtenidos antes y después de la inducción de choque térmico a 42°C.

Resultados. El extracto de queratinocitos de piel sana fue reconocido por ambos sueros anti-estreptococo, sin que hubiera diferencias significativas entre ellos. El suero de los pacientes psoriásicos no mostró reconocimiento de los antígenos presentes en el extracto de queratinocitos.

Conclusiones. Aparentemente hay antígenos epidérmicos constitutivos con reacción cruzada hacia *S. pyogenes*. A pesar de que se ha reportado la presencia de autoanticuerpos con reactividad cruzada hacia *S. pyogenes* en el suero de pacientes psoriásicos, nuestros resultados sugieren que el blanco de esos autoanticuerpos podría expresarse durante el desarrollo de la enfermedad pues no están presentes en la piel sana. ***(Rev Biomed 2003; 14:69-74)***

Palabras clave: Psoriasis, Autoinmunidad, Autoantígenos, *Streptococcus pyogenes*.

INTRODUCTION.

Psoriasis is a chronic skin disease that has been suggested to be of autoimmune nature. It is characterized by the presence of maculo-papulous, erithematous lesions that are covered by adherent scales (1-7). The etiopathogenesis of the disease remains unknown, but it is considered as a polygenetic and multifactorial disease (8-9). Nevertheless, *Streptococcus pyogenes* throat infection is the only factor convincingly associated with the onset or exacerbation of the disease (10). It has been reported that a great proportion of psoriatic patients have had a streptococcal throat infection approximately two weeks before the onset of the epidermal lesions. Patients have high antistreptolysine titers and *S. pyogenes* can be isolated from their throat cultures in nearly 80% of them (1, 4, 10-12). It has also been found that the 14, 60; and 70 kDa proteins from *S. pyogenes* appear to be immunodominant antigens for humoral immune response in those patients. It is noticeable that these proteins are coincident in molecular mass with the streptococcal heat shock proteins (HSP) (13). As demonstrated by indirect immunofluorescence (13) and by immunoperoxidase (14), there are antigens in keratinocytes from psoriatic lesional skin that are recognized by mice or rabbit antisera and by monoclonal antibodies to *S. pyogenes*. Skin from psoriatic patients without histological lesion or from healthy donors show negative reactions. In addition, sera from psoriatic patients have antibodies that are able to recognize some antigens in keratinocytes from epidermal lesions (13-14). Psoriatic patients also have antibodies to 48-50 kDa cytokeratins which are overexpressed in this kind of lesions (15).

MATERIAL AND METHODS.

Antigens.

Streptococcal antigens. A *Streptococcus pyogenes* M5 strain, obtained from the CDC, Atlanta, GA, was cultured as previously described (13-14). Briefly, the bacteria were cultured in Todd-Hewitt broth with 1% peptone at 37°C for 18 h. To obtain the extract, the bacterial mass was sonicated at 180 W in 1-minute intervals for 20 minutes, in an ice bath. Finally, cellular walls were eliminated by centrifugation and the supernatant was called Total Soluble Extract of *Streptococcus pyogenes* at 37°C (TSE37Sp). In addition, we induced heat shock in *S. pyogenes* to increase the expression of HSP. That is, the bacteria cultured as above were additionally incubated for 16

h at 42°C, and then sonicated, to obtain what we called TSE42Sp. In both cases, protein concentration was determined by Bradford's method (16).

Epidermal antigens.

Six punch skin biopsies (8 mm) were obtained from healthy donors. Skin biopsies from the forearm were obtained with xilocaine-1% epinephrine anesthesia. From this material, an antigenic extract was obtained as previously described (17). Briefly, biopsies were washed with Dulbecco´s Modified Essential Medium (DMEM) with antibiotics. Dermis was removed by treatment with colagenase in DMEM at a final concentration of 200U/mL for 16 h at 37°C. The resultant epidermal sheets were washed by centrifugation to eliminate the enzyme. The sheets were cut in small pieces and treated overnight with 0.25% trypsine in versene at 4°C. The keratinocyte suspension obtained was washed by centrifugation and sonicated at 110 W at 15-second intervals for 15 min in an ice bath. The supernatant was called Total Soluble Extract of Keratinocytes (TSEKC). Protein concentration was determined by Bradford's method (16).

Patients.

Sera obtained from a group of 26 clinically diagnosed psoriatic patients, all of them with the plaque form of the disease, at the Hospital General de Zona No. 1, Instituto Mexicano del Seguro Social (IMSS), Campeche, and sera from 26 non-psoriatic subjects were analyzed by ELISA against TSEKC antigens. Age and sex of the patients are shown in table 1. Selection of patients and controls was done by the dermatologist at the clinical institution following the clinical criteria established for the diagnosis (18).

Table 1
Age and sex of patients included in the study.

Age (years)	Women	Men	Total
20-30	1		1
31-40	2	1	3
41-50	2	4	6
51-60	3	8	11
>60		5	5
Total	8	18	26

Antisera.

Two groups of four 8-week old female NIH mice were immunized with TSE37Sp or TSE42Sp. Four doses of 100 µg of protein (1.0 mg/mL) were given intraperitoneally on days, 0, 5, 10, and 15. The first dose was given in incomplete Freund´s adyuvant and the others in 0.85% saline solution. Mice were bled on day 17 from the tail vein. A pool of sera from each group of mice was obtained and titrated by dot enzyme-linked immunosorbent assay (ELISA) (19-20)

Immunoassay analysis.

Antibody responses to epidermal antigens were evaluated by ELISA. The ELISA plates were coated with the TSEKC in a 1.0 mg/mL protein concentration. Sensitization was carried out overnight at 4°C in pH 9.6 carbonate-bicarbonate buffer. Non-specific binding sites were blocked with 3.0% non-fat milk in PBS (PBS-G). Then the serum of each patient or the anti-streptococcal antisera at a 1:100 dilution in PBS-G was added to the corresponding well, incubated for 1 h at 37°C and washed with 0.05 % $Tween_{20}$ in PBS (PBS-T). Plates were incubated 1 h with peroxidase-conjugated goat anti Human IgG or peroxidase-conjugated goat anti mouse IgG (Dako Corporation) and washed again. Finally, the reaction was developed with 0.03% H_2O_2–o-phenylendiamine (Sigma Chemical, Co.) in citrate-phosphate, pH 5.0 buffer. Incubation in the dark at room temperature for 15 minutes was done and the reaction was stopped with 50 µL of 8.0 N sulfuric acid. Absorbance was read at 492 nm in a Multiskan Ascent microplate reader (Labsystems) (19-20).

Statistical analysis.

When recognition of epidermal antigens was detected, the absorbance results in the ELISA assays were compared by the *student's t* test with a 95% interval of confidence.

RESULTS.

Neither the sera from psoriatic patients nor those from healthy subjects showed recognition of antigens in the healthy skin derived extract. In all cases,

absorbances at 492 nm were less than 0.1 ($A4_{492}$= 0.083 +/- 0.016). Nevertheless, a strong recognition was evident when those sera were tested against streptococcal extracts (data not shown). As can be seen in figure 1, both anti-streptococcal sera recognized the epidermal extract (TSEKC). When we analyzed the recognition of TSEKC by the anti-TSE37Sp sera, the mean absorbance value was 0.197, while anti-TSE42Sp showed a mean value of 0.188 when tested. No significant differences were found when the *student's t* test was done (p=0.184).

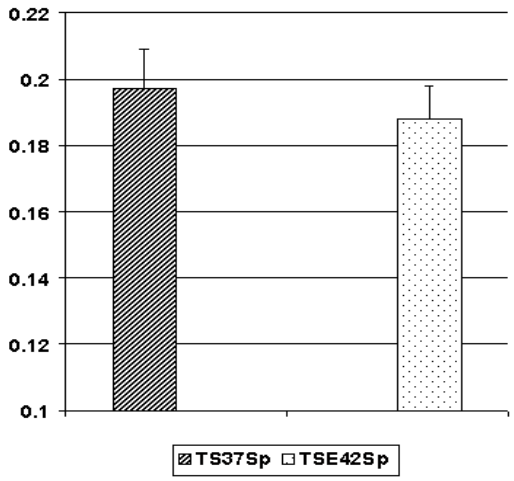

Figure 1.- Recognition of epidermal antigens by anti-streptococcal antisera. ELISA plates were sensitized with a total antigenic extract of healthy-skin-derived keratinocytes, and the anti TSE37Sp and TSE42Sp sera were tested as described in material and methods. Mean absorbance values and standard deviation (bars) of three assays in triplicate are shown. No significant differences were reached (p=0.184).

DISCUSSION.

In this work we found no recognition of an antigenic extract of skin biopsies from healthy (non-psoriatic) donors by sera from psoriatic patients or the controls included. These results are in agreement with the previous reports indicating that the sera from psoriatic patients have autoantibodies that recognize antigens in the lesional-skin-derived keratinocytes, without recognition in healthy skin even if it was obtained from a non-lesional region of the same psoriatic patient (13-14). Moreover, as control, we also looked for the presence or cytokeratins in our epidermal extracts. We found that monoclonal antibodies to keratins of a molecular mass between 50 and 68 kDa were present in our antigenic extracts (data not shown). It is important to note that, as shown by Aoki *et al.* cytokeratins between 48 and 50 kDa are mostly recognized by psoriatic patients (15). Even more, those proteins are overexpressed in the skin from the lesions in these kind of patients. Taking our findings into account, we may suggest that the epidermal antigens from lesions that are recognized by autoantibodies in psoriatic patients, are not skin-constitutive antigens, but are synthesized after the pathologic phenomenon has been initialized.

In addition, we analyzed the presence of skin antigens that cross-react with streptococcal antigens. The mice sera to TSE37Sp and to TSE42Sp recognized antigens in the extracts from healthy-skin-derived keratinocytes with no significant differences between them. These data are in accordance with those that indicate that *S. pyogenes* cross-reacts with different human tissues, including skin (21-23). Swerlick *et al.*, and Cunningham and Swerlick, using indirect immunofluorescence, have demonstrated that monoclonal antobodies against *S. pyogenes* recognize antigens both in healthy or psoriatic human skin (22, 24). Furthermore, It has been reported that lesional skin shows stronger and more extensive reactions with anti-M protein antibodies than does that from healthy subjects (25). Nevertheless, we have shown that only lesional skin is recognized by a policlonal antiserum to TSE37Sp (14). Taking this into consideration, we could propose that healthy-skin-derived keratinocytes have antigens that cross-react with antigens from *Streptococcus pyogenes*, as reported. Although we have not demonstrated so, those cross-reactive antigens could correspond to the pso p27 antigen described by Iversen *et al.* They have described scale antibodies in psoriatic patients that react to it (26). In addition, these results agree with the concept stating that the susceptibility to the development of psoriasis is related to the appearance of abnormal or occult epitopes in the skin from the patients (21).

We can conclude that the autoantigens that

appear to be cross-reactive with streptococcal antigens and involved in the pathogenesis of psoriasis are probably different from constitutive cross-reactive antigens, and only expressed in lesional skin after the early development of the disease. So, it is possible that not only genetic background and streptococcal throat infections but also another environmental trigger factor is involved in the pathogenesis of the disease in a Koebner phenomenon way (27).

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