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LETTERS TO THE EDITOR

Antibodies to the 90 kDa Heat Shock Protein in Chronic Plaque Psoriasis

Sir,

The response of eukaryotic cells to elevated temperature and many other unrelated forms of stress, such as ischaemia, inflammation, infection and free radicals, involves the expression of a set of proteins commonly referred to as heat shock proteins (HSPs) or stress proteins (1, 2), which seem to protect cells from some of the effects of stresses. Of the multiple families of HSPs, proteins from the HSP90 and HSP70 families are the best studied in mammalian cells (1, 2). Some heat shock proteins have a role as molecular chaperones, but HSP90 is also present in the cytoplasm of unstressed cells and is found in transient association with steriod hormone receptors, an oncogene product pp60 v-src, initiation factor (eIF-2α) kinase and F-actin (1). The inducible HSP70 is believed to play a role in cell repair by helping in protein renaturation (1). We report here the apparent presence of serum antibodies to stress proteins in plaque psoriasis patients, and the expression of stress protein mRNAs in inflammatory lesional psoriatic scales.

MATERIALS AND METHODS

Blood samples and scale material from skin lesions were taken from 4 patients with chronic plaque type psoriasis (2 men and 2 women, age range 22–65 years), attending the outpatient dermatology clinic at Leeds General Infirmary. The duration of disease varied widely (5 to 28 years). None of the patients was on conventional treatment for 2–3 weeks prior to specimen collection. Control blood samples were taken from 4 healthy volunteers with no clinical evidence of skin or other diseases. The serum was separated by centrifugation and then kept frozen in aliquots at −20°C until assayed. Samples of keratotomed epidermis from human skin discarded at surgery were used as normal controls in this study. Total RNA was isolated from the scale material and keratotomed epidermis by the guanidinium isothiocyanate method of Chirgwin et al. (3). RNA samples (10 mg) were electrophoresed on a 2% agarose-formaldehyde gel and then transferred to Zeta-probe blotting membrane (Bio-Rad, Richmond, USA). The membranes were prehybridised at 42°C for 4 h and then hybridised for 10 h with denatured HSP90α and HSP70B' cDNA probes (StressGen, Victoria, Canada), labelled by the random priming method. The membranes were then washed and exposed to Kodak X-ray film at −70°C.

Primary cultures of normal human keratinocytes were obtained from foreskins (4) and grown at 37°C to 90% confluency in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Second passage cells were heated to 44°C for 1 h by submersion of the culture dishes in a water bath and were then allowed to recover at 37°C for 2 h. Total cellular proteins were extracted in SDS lysis buffer (2% SDS, 10% glycerol, 3% 2-mercaptoethanol, 50 mM Tris-HCl, pH 6.8) and the protein content was determined spectrophotometrically (5). This protein mixture was used as stress protein antigens for the analysis by immunoblotting of subject sera.

Samples of antigen (15 μg) were loaded into wells on 8.5% polyacrylamide gels. After electrophoresis and transblotting (6, 7) onto nitrocellulose membranes, the membranes were blocked in 3% bovine serum albumin in buffered saline (0.9% NaCl and 10 mM Tris-HCl, pH 7.4) at 4°C overnight. The nitrocellulose sheets were then incubated at room temperature for 2 h with the human serum samples diluted 1:40 in buffered saline containing 3% bovine serum albumin and 0.05% Tween 20. After washing five times for 25 min in 0.9% saline/0.05% Tween 20, the nitrocellulose sheets were incubated for 1 h with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) at a dilution of 1:500. After another washing, the membranes were incubated for 5 min at room temperature with staining buffer (100 mM Tris-HCl, pH 9.5 containing 100 mM NaCl and 5 mM $MgCl_2$) containing a mixture of 66 μl of NBT (50 mg/ml in 70% DMF) per 10 ml and 33 μl of BCIP (50 mg/ml in 70% DMF) per 10 ml. The reaction was stopped by washing the membranes in distilled water. Prestained molecular weight markers were used as standards.

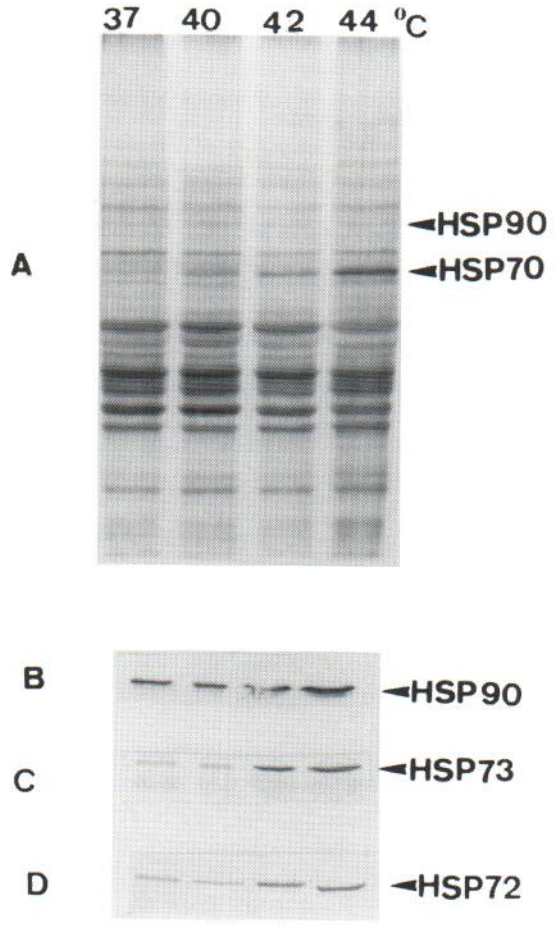

Fig. 1. Expression of stress proteins following hyperthermia in cultured keratinocytes. (A) Autoradiograph of SDS-PAGE (8.5% polyacrylamide) of protein extracted from keratinocytes that had been heat-shocked at the temperatures indicated for 1 h and labelled with [^{35}S] methionine for 2 h (37°C is the control). Each track was loaded with equal numbers of cpm. *Arrows* indicate the bands due to the heat shock proteins, HSP90 and HSP70. (B) The immunoblot of the above samples using a monoclonal antibody specific for HSP90. (C) Immunostaining using a monoclonal antibody specific for the constitutive HSC73 and the inducible 72 kDa. (D) The same samples except that they were stained with a monoclonal antibody specific for the inducible HSP72.

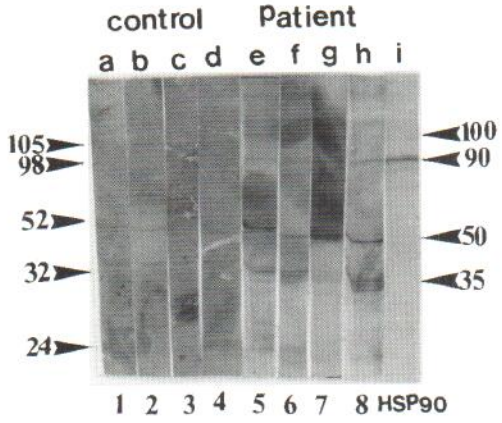

Fig. 2. Antibodies to heat-shocked keratinocyte antigens in normal controls and in patients with plaque psoriasis. Four sera from controls and 4 from psoriatic patients were used in the immunoblotting analysis to recognise antigens of total cellular extract from cultured keratinocytes heat-shocked at 44°C for 1 h. Each lane was loaded with 15 μg of total cellular protein extract. Lanes *a* to *h* represent patients and lane *i* represents the immunostaining of HSP90 using the monoclonal antibody (9D2 which is specific for HSP90). The molecular masses of the mol.wt. markers and the polypeptides recognised by the sera antibodies are given in kDa on the left and right sides, respectively.

RESULTS

Cultured normal keratinocytes that had been heat-shocked for 1 h at 44°C showed a striking increase in the levels of HSP90 and especially HSP70 (Fig. 1). These extracted proteins were used as the source of antigens and were separated by SDS-PAGE and immunoblotted with sera from patients. Serum samples from normal control patients showed detectable IgG responses to bands at 35 and 50 kDa (Fig. 2), but when sera from patients with chronic plaque psoriasis were tested, two antigenic bands of 100 and 90 kDa in addition to those recognised by control sera were identified (Fig. 2). The band at 90 kDa was the most strongly reacting with the sera of all psoriatic patients containing an IgG, while the band at 100 kDa was observed in the sera of only 2 patients. We assume that the 90 kDa psoriasis autoantibody target was the 90 kDa stress protein based on the positional comparison between the HSP90 band detected by the monoclonal antibody 9D2 (which recognises the HSP90), and the protein markers.

The transcriptional levels of both HSP90α and HSP70B' genes in psoriatic scale and normal skin keratotome samples were almost identical (Fig. 3), whereas their levels were clearly elevated in heat-shocked cultured keratinocytes. In fact, HSP90 mRNA is present at fairly high levels in normal skin and scale material, but in contrast little HSP70 mRNA was present.

DISCUSSION

Antibody responses to proteins extracted from heat-shocked keratinocytes were present in both controls and in plaque psoriasis patients. The recognition patterns of IgG antibody in normal sera and patients' sera were found to be slightly variable by the immunoblotting technique, and a cluster of bands at 35 and 45 kDa was the common occurrence of IgG antibodies. The IgG antibody to the 90 kDa polypeptide was demonstrated in a disease-specific manner, and no IgG band corresponding to this polypeptide was detected in the normal sera tested. The protein is assumed to be the 90 kDa heat shock protein because its molecular mass corresponded with the band that stained by a monoclonal antibody specific for HSP90 (Fig. 2, lane i). Because this study used keratinocytes, it was of interest to address the questions of the origin of this stress protein and how the autoantibody against it is raised. It is likely that serine proteases (human leukocyte elastase and cathepsin) of neutrophils which cause proteolytic tissue damage may be the factor in the development of autoimmune reactivity. The activities of these enzymes were elevated in the skin in psoriasis, where neutrophils infiltrate the epidermis (8). Also it is well known that HSP90 is present in considerable amounts in both stressed and unstressed keratinocytes and in dermal fibroblasts (9). Taken together, this suggests that the HSP90 released as a result of cellular damage may leak into the bloodstream, leading to the formation of autoantibodies to this antigen. The presence of autoantibodies against HSP90 is also found in systemic lupus erythematosus (10). However, Latchman (11) has cautioned against drawing conclusions about autoreactivity, since some of the observations may be explained by the presence of mycobacterial antibodies in sera. Nevertheless, this does not explain why the antibodies only seem to be observed in psoriasis sera. The present observation of a serum antibody response of psoriasis patients to HSP90 suggests that shared or common antigens may occur among various protein products from epithelial cell destruction, resulting in immunoreactivity that does not reflect the true presence of antibodies to HSP90. According to this view, the presence of antibodies in psoriasis patients may reflect non-specific immune activation, confirming the fact that autoimmune reactivity by itself does not necessarily lead to autoimmune disease. Individu-

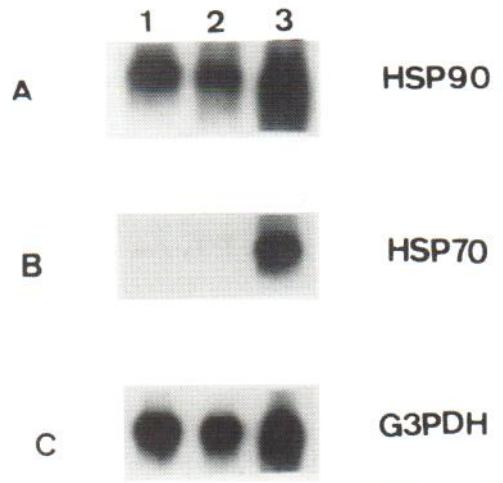

Fig. 3. Northern blot analysis of mRNAs for HSP90α and HSP70B' in psoriatic and normal epidermis and in heat-shocked cultured keratinocytes. Equal amounts of total RNA (10 μg) were applied to each lane, extracted from (1) normal skin; (2) psoriatic scale; (3) keratinocytes heat-shocked at 44°C for 1 h. Levels of cellular HSP90α and HSP70 mRNA were determined using the [^{32}P] cDNA probe encoding HSP90 and HSP70B'. The hybridisation signal was detected by autoradiography. The G3PDH probe was used as a control to compare RNA loading in each lane.

als may have antibodies or T cells that can interact with epitopes present in their own tissues without any signs of disease (12).

A somewhat unexpected finding of this study was that HSP mRNA was detectable in psoriatic scales although this may be the result of the parakeratotic character of psoriatic epidermis. These nucleated horny cells are assumed to represent partly differentiated quiescent or senescent cells that may be metabolically active, but which do not grow or undergo any of the events and processes of the cell cycle. Therefore, the low levels of HSP mRNAs found by Northern blot from psoriatic scales may be ascribed either to the dysfunction in the signalling mechanism that occurs at the level of the gene with senescent cells (13) or to the decreased synthesis of HSPs following the induction of differentiation (14).

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