Immunolocalisation of the janus kinases (JAK)—signal transducers and activators of transcription (STAT) pathway in human epidermis

HAJIME NISHIO, KIYOSHI MATSUI, HIROKO TSUJI, AKIYOSHI TAMURA AND KOICHI SUZUKI

Osaka Medical College, Department of Legal Medicine, Takatsuki, Japan

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ABSTRACT

The janus kinases (JAK) and signal transducers and activators of the transcription (STAT) pathway have been shown to be activated by a number of cytokines or growth factors and to play significant roles in the differentiation of various cell types. In the present study, we investigated the distribution of the JAK–STAT pathway using immunohistochemistry in the human epidermis. Each element of the pathway showed abundant and differential expression in the epidermis. The differential distribution of the elements was most strikingly observed in the horny keratinised cell and granular layers of the epidermis. JAK2, JAK3, STAT1 and STAT5 were expressed in high amounts, and JAK1, TYK2, STAT2, STAT3, STAT4 and STAT6 to a much lesser extent in the horny cell layer. JAK3, TYK2, STAT2, STAT3, STAT4 and STAT6 were more abundantly expressed in the granular layer than the lower layers of the epidermis. JAK1, STAT1 and STAT5 were expressed at almost the same levels in the various layers of the epidermis. These results show that elements of the JAK–STAT pathway are abundantly and differentially expressed in the epidermis. It is suggested that each element of the pathway may play a role at a distinct stage of keratinocyte differentiation.

Key words: Skin; keratinocyte differentiation.

INTRODUCTION

The janus kinases (JAK) and signal transducers and activators of the transcription (STAT) signalling pathway have been shown to be activated by a number of cytokines, growth factors, and oncogenic tyrosine kinases (Ihle, 1995). JAKs associate with the intracellular domains of particular receptors and become activated by ligand binding to the receptors at the cell surface. Activated JAKs consecutively phosphorylate STATs at distinct tyrosine residues. The tyrosine-phosphorylated STATs leave the receptor complex, translocate to the nucleus, and promote the transcriptional activation of ligand-inducible genes (Darnell, 1997). To date, 4 members of the JAK family (JAK1, JAK2, JAK3 and TYK2) and 7 of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6) have been identified (Ihle, 1995). The pathway has been demonstrated to play significant roles in determining differentiation processes of a variety of cell types, including myeloid leukaemia cells (Minami et al. 1996; Nakajima et al. 1996; Yamanaka et al. 1996), murine mammary epithelial cells (Liu et al. 1997), helper T cells (Shimoda et al. 1996; Takeda et al. 1996), murine haematopoietic cells (Rane & Reddy, 1994) and rat hepatocytes (Runge et al. 1998).

The epidermis provides a useful model for investigating the differentiation process. In the epidermis, the outer (horny cell) layers of terminally differentiated cells are repopulated from a basal layer of proliferating keratinocytes. The proliferating keratinocytes are released from the substratum, migrate outwards and enter a differentiated pathway. STAT3 has already been shown to play an important role in keratinocyte differentiation (Hauser et al. 1998).

Correspondence to Dr Koichi Suzuki, Department of Legal Medicine, Osaka Medical College, 2–7 Daigaku, Takatsuki, 569-8686, Japan. Tel.: +81 726 831221; fax: +81 726 846515; e-mail: leg010@art.osaka-med.ac.jp

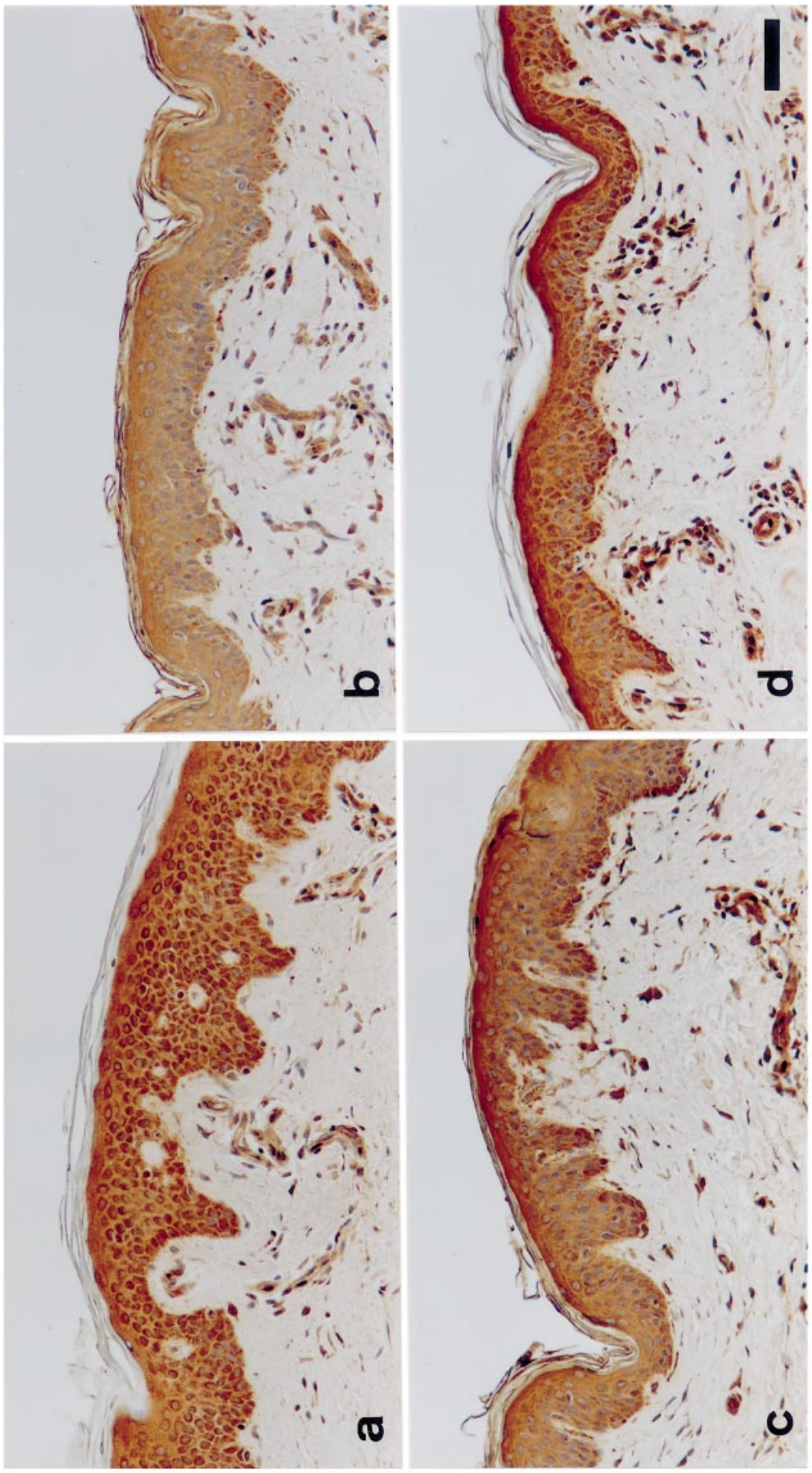

Fig. 1. Immunolocalisation of JAK1 (*a*), JAK2 (*b*), JAK3 (*c*) and TYK2 (*d*) in the human epidermis. Bar, 80 μm. Haematoxylin counterstain.

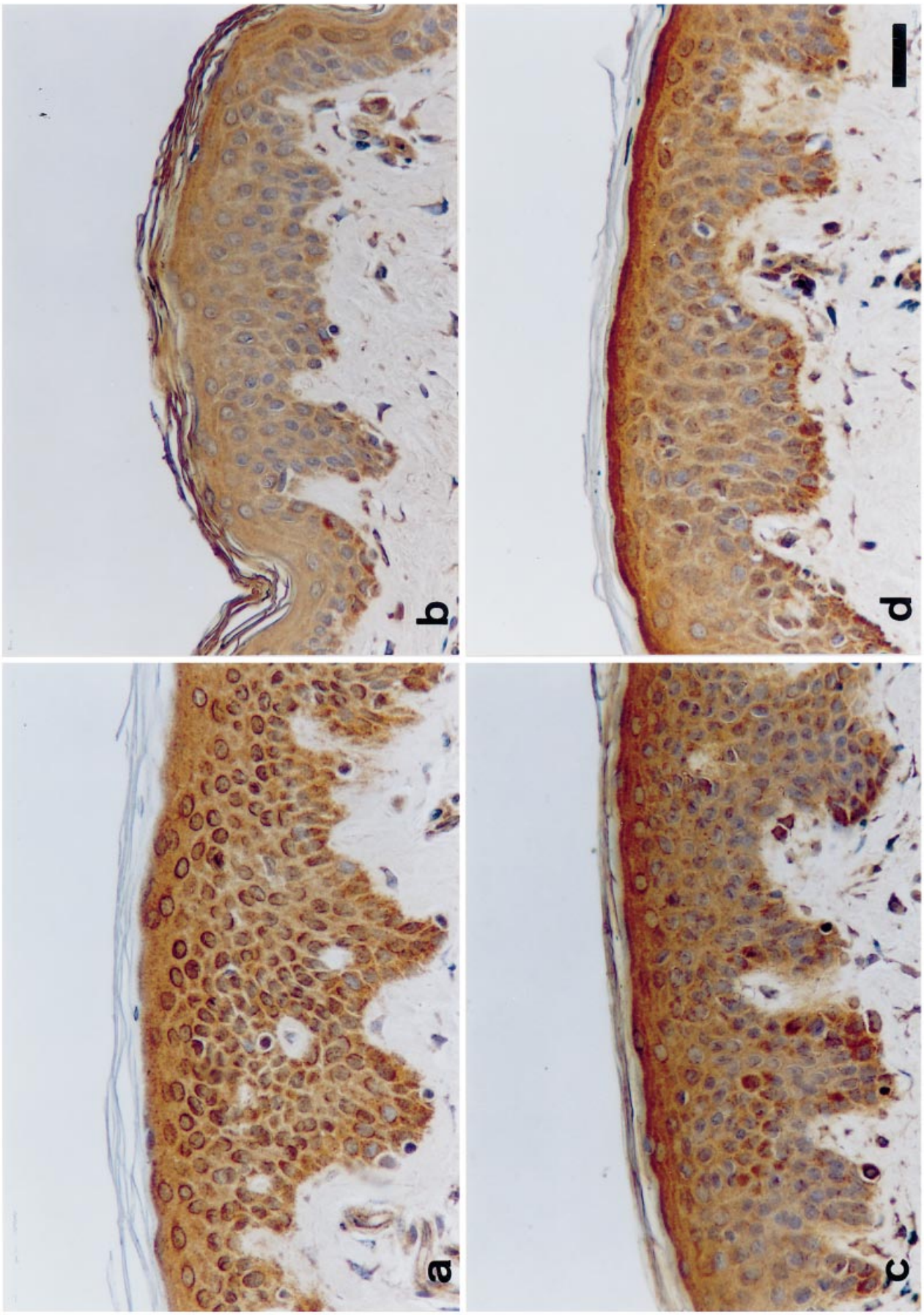

Fig. 2. High magnification photomicrographs of localisation of JAK1 (*a*), JAK2 (*b*), JAK3 (*c*) and TYK2 (*d*) in the human epidermis. Bar, 40 μm. Haematoxylin counterstain.

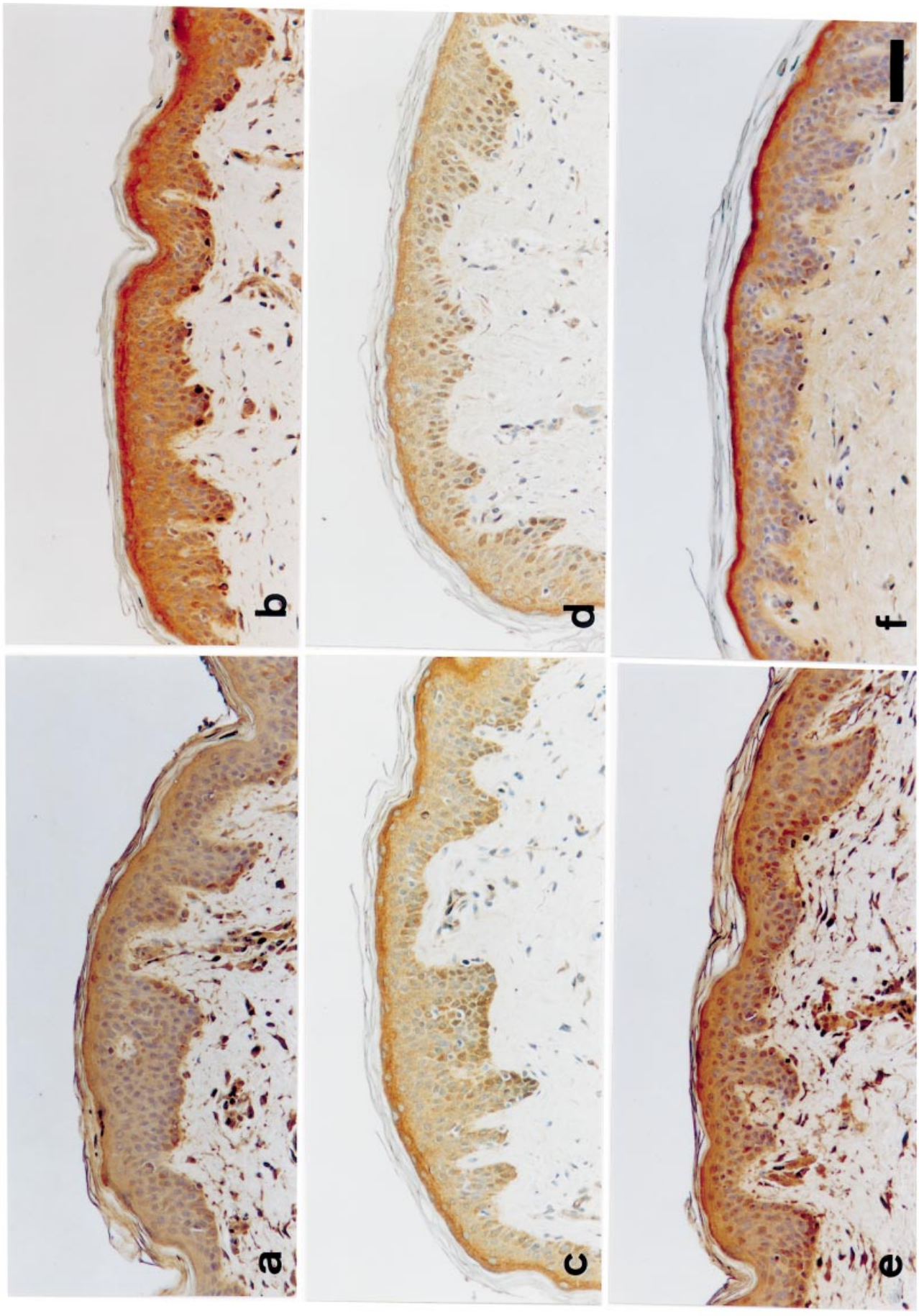

Fig. 3. Immunolocalisation of STAT1 (*a*), STAT2 (*b*), STAT3 (*c*), STAT4 (*d*), STAT5 (*e*) and STAT6 (*f*) in the human epidermis. Bar, 80 μm. Haematoxylin counterstain.

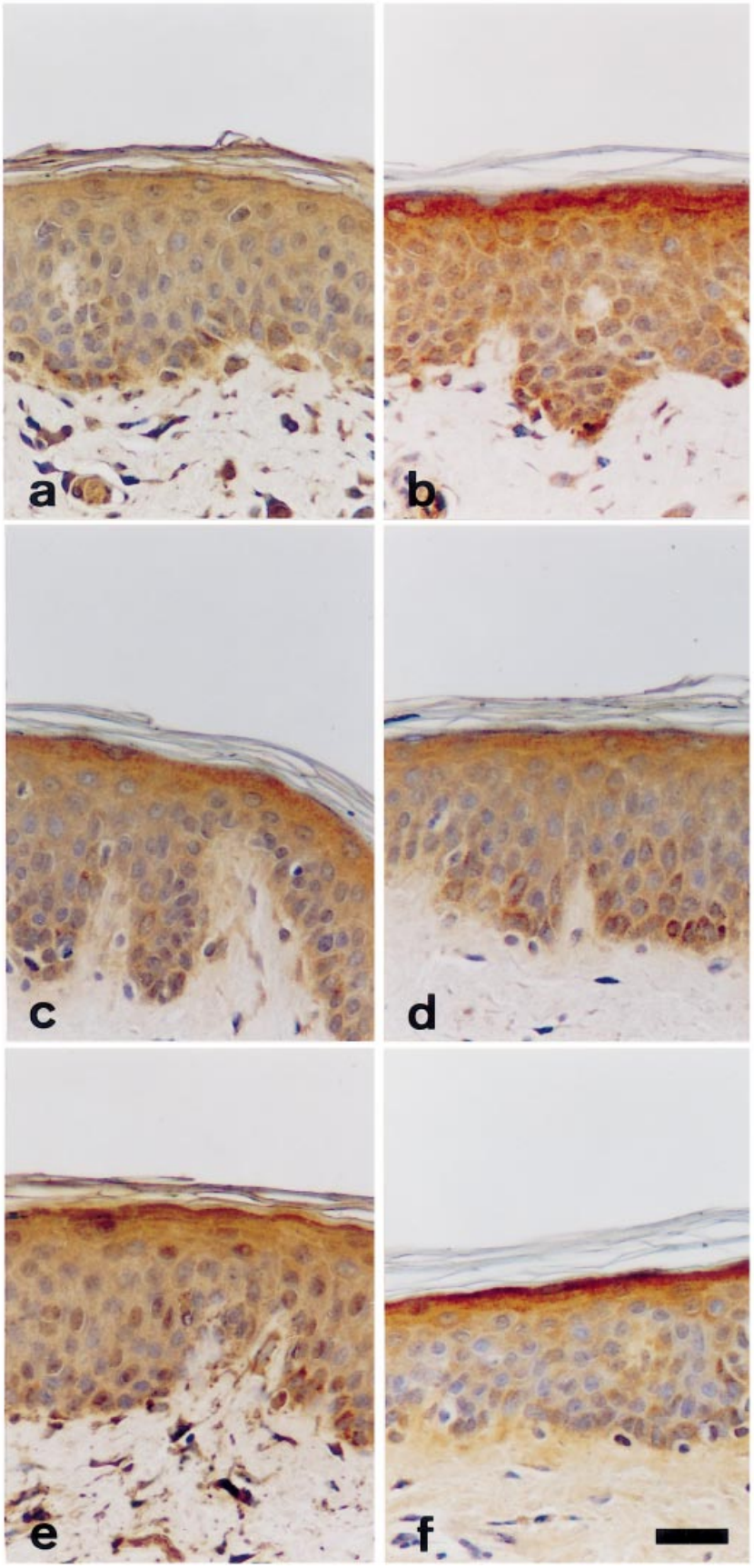

Fig. 4. High magnification photomicrographs of localisation of STAT1 (*a*), STAT2 (*b*), STAT3 (*c*), STAT4 (*d*), STAT5 (*e*) and STAT6 (*f*) in the human epidermis. Bar, 40 μm. Haematoxylin counterstain.

However, the role of other members of the pathway in keratinocyte differentiation is not well understood, and there is a lack of information on the localisation of elements of the pathway in the epidermis.

In the present study, we performed by immunohistochemical methods a systematic survey of the localisation of the elements in human epidermis and demonstrated their abundant and differential expression.

MATERIALS AND METHODS

Tissue

Epidermis and the underlying dermis were obtained from the abdominal skin of cadavers subjected to judicial autopsies performed at the Institute of Legal Medicine, Osaka Medical College, Osaka, Japan. Autopsies were carried out within 24 h of death in all cases on subjects ranging in age from 23–76 (mean 46.8) and of both sexes (4 males, 3 females).

Antibodies

All antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). JAK1(HR-785), JAK2(C-20) and TYK2(C-20) antibodies were rabbit polyclonal antibodies. JAK3(N-15) antibody was a goat polyclonal antibody. STAT1(E-23), STAT2(C-20), STAT3(H-190), STAT4(H-119) and STAT6(M-200) antibodies were rabbit polyclonal antibodies. STAT5(C-17-G) antibody which recognised both STAT5a and STAT5b was a goat polyclonal antibody.

Immunohistochemistry

The epidermis and the underlying dermis were removed and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, and the specimens were processed for paraffin embedding. Sections (4 μm) were cut and placed on gelatin-coated glass slides. Immunostaining was carried out using the avidin–biotin–complex (ABC) method with peroxidase as label (Vectastain ABC Elite Kit; Vector Laboratories, Burlingame, CA) according to the supplier's instructions. Following blocking with normal serum, sections were incubated with the primary antibodies (1:100 dilution) for 16 h at 4 °C, and with the secondary biotinylated antibodies for 1 h at room temperature in a humidified chamber. The ABC complex was visualised by incubating the sections at room temperature in the presence of 3-3′-diaminobenzidine and 0.005% H_2O_2, and counterstained for 45 s with haematoxylin. Control experiments were carried out using preimmune IgG as a primary antibody at an equivalent dilution. Preimmune rabbit IgG was used as the primary antibody for the JAK1, JAK2, TYK2, STAT1, STAT2, STAT3, STAT4 and STAT6 antibody staining procedures. Preimmune goat IgG was used as a primary antibody for the JAK3 and STAT5 antibody staining procedures.

RESULTS

Expression of the JAK family in the human epidermis

We investigated the immunohistochemical distribution of the JAK family in the human epidermis where each member showed a distinct localisation. Strong immunoreactivity with JAK1 antibody was detected in the epidermis (Figs 1*a*, 2*a*) but the horny cell layer was not stained. The staining pattern with TYK2 antibody was similar to that with JAK1 antibody, though the immunoreactivity was more strongly detected in the granular layer than in lower layers of the epidermis (Figs 1*d*, 2*d*). Immunoreactivity with JAK2 antibody was found throughout the epidermis with the horny cell layer being strongly stained by the antibody (Figs 1*b*, 2*b*). Immunoreactivity with JAK3 antibody was almost identical to that with JAK2 antibody (Figs 1*c*, 2*c*). Control incubations with preimmune IgG did not result in any significant staining (Fig. 5).

Expression of the STAT family in the human epidermis

We further investigated whether the STAT family, which is a downstream substrate of the JAK family, also showed distinct localisation in the epidermis. The immunoreactivity with STAT2 antibody was more strongly detected in the granular layer than in lower layers of the epidermis (Figs 3*b*, 4*b*). The horny cell layer was not stained by the antibody. Staining patterns with STAT3, STAT4, and STAT6 antibodies were almost identical to those with STAT2 antibody (Figs 3*c*, *d*, *f*; 4*c*, *d*, *f*). Immunoreactivity with STAT1 antibody was detected in the entire epidermis with the horny cell layer being strongly stained (Figs 3*a*, 4*a*). The staining pattern with STAT5 antibody was almost the same as to that with STAT1 antibody (Figs 3*e*, 4*e*). Control incubations with preimmune IgG did not result in any significant staining (Fig. 5).

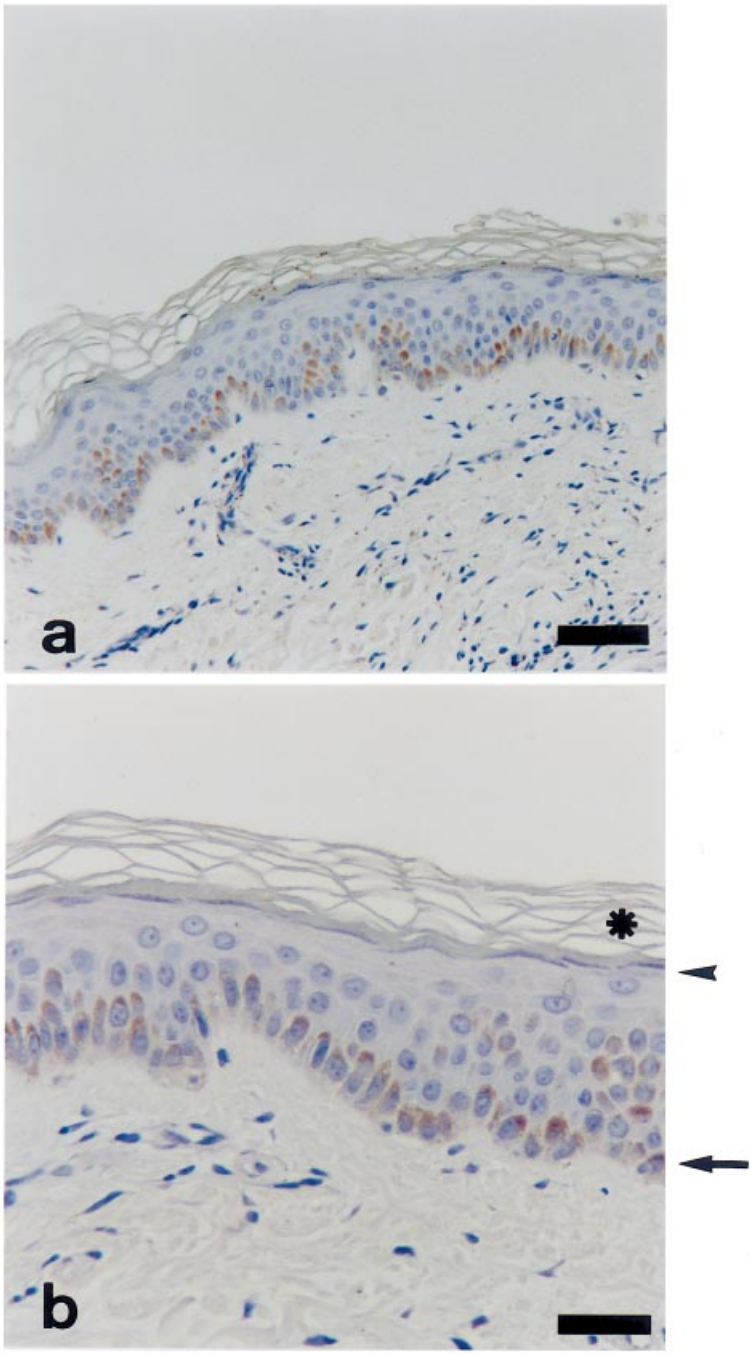

Fig. 5. Control staining using preimmune rabbit (*a*) and goat IgG (*b*) in the human epidermis. Haematoxylin counterstain. Asterisk, horny cell layer; arrow head, granular layer; arrow, basal layer. Bars: 80 μm (*a*); 40 μm (*b*).

DISCUSSION

We previously demonstrated that phosphotyrosine-containing proteins were abundantly expressed in Hassall's corpuscles of the human thymus (Nishio et al. 1999) and that the JAK–STAT pathway was, in part, responsible for that expression (Nishio et al. 2000). Hassall's corpuscles are a unique feature of the medulla of the thymus and are representative of the terminal stages of thymic medullary epithelial maturation (Nicolas et al. 1989). Because of the abundant expression of the various elements of the pathway in Hassall's corpuscles, our previous results strongly suggested that the pathway may be involved in the differentiation process of the thymic medullary epithelium (Nishio et al. 2000). Interestingly, Hassall's corpuscles have been reported to possess similarities to epidermis on the basis of an electron microscopical and histochemical study (Gaudecker & Schmale, 1974). Furthermore, Patel et al. (1995) compared the phenotype of thymic epithelial cells to 83 other cell lines studied in human leukocyte differentiation

antigens (HLDA-V), and found that thymic epithelial cells were strikingly more similar to epidermal keratinocytes than to any of the other cell types tested. These reports indicated that there are substantial similarities between Hassall's corpuscles and epidermis, which led us to investigate whether elements of the pathway may play a role in the differentiation process of keratinocytes as well as in that of thymic epithelial cells.

Recently, STAT3 has been shown to play an important role in the differentiation process of keratinocytes (Hauser et al. 1998). According to this report, STAT3 is activated in keratinocytes which are in the stage of growth arrest and differentiation. In addition, induction of STAT3 is not observed in an immortalised keratinocyte cell line, and when p27kip1 which is an important factor for growth arrest was inhibited, there was a loss of STAT3 activation and keratin 1, a differentiation specific marker. Thus these findings strongly suggest that STAT3 plays a role in keratinocyte differentiation. The roles of other elements of the pathway in differentiation of keratinocytes have not been elucidated. However, since some elements have been identified to play a role in the differentiation process of various cell types, there might be the possibility that some elements other than STAT3 are involved in the differentiation process of keratinocytes.

In the present study, we found that various elements of the JAK–STAT pathway are abundantly and differentially expressed in the epidermis, indicating that the expression of elements of the pathway is regulated during keratinocyte differentiation. To our knowledge, this is the first report demonstrating a systematic survey of the localisation of elements of the JAK–STAT pathway in the human epidermis. STAT3 has been observed to be expressed abundantly in the upper epidermis where more differentiated keratinocytes are located, which may support the previous report of its involvement in keratinocyte differentiation (Hauser et al. 1998). The distribution patterns of STAT2, STAT4 and STAT6 in the epidermis were almost identical to that of STAT3. TYK2 also showed similar staining pattern to that of STAT3. Judging just from the immunohistochemial findings, these elements may also be involved in the differentiation process of keratinocytes as well as STAT3, although further study is necessary to clarify their exact roles in keratinocyte differentiation.

The functional significance of the differential localisation of JAK and STAT in the epidermis has not been clarified in the present study. However, since each cytokine has been reported to utilise a distinct element of JAK and STAT as the intracellular signal transduction, our results may suggest that each cytokine exerts its action in keratinocytes which are in a different stage of differentiation.

From research in the field of immunodermatology, there is increasing evidence that the skin immune system is regulated by a complex pattern of cytokines. Any imbalance of this network could result in a profound dysregulation, finally expressed as a specific disease (Kapp, 1993). Recently, STAT1 activation has been reported to show a reduced response to gamma-interferon (γ-IFN) in keratinocytes from psoriasis, which is a chronic inflammatory skin disease with the histopathological characteristics of (1) epidermal proliferation and disturbed differentiation and (2) an inflammatory infiltrate (comprising neutrophils and T cells) (Jackson et al. 1999), suggesting that abnormal signalling in the JAK–STAT pathway in response to γ-IFN occurs in psoriatic keratinocytes. Because of the importance of JAK–STAT pathway in cytokine-mediated signalling, the distinct localisation pattern of the elements of the JAK–STAT pathway demonstrated in the present study may be important for structural and functional homeostasis of normal epidermis.

In conclusion, the present study has demonstrated the localisation of the elements of JAK–STAT pathway in normal epidermis, and has provided evidence that the various elements of the pathway are abundantly and differentially expressed in the epidermis, indicating that their expression is regulated during keratinocyte differentiation.

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