Reactivity of T lymphotropic retrovirus antibody (12/1-2) in man: comparison of epidermis with other epithelial cells

ELISABETH RALFKIAER,*† KAREN AF PULFORD,* KC GATTER,*
GUNHILD LANGE WANTZIN,‡ DY MASON*

From the *Nuffield Department of Pathology, John Radcliffe Hospital, University of Oxford, and the Departments of †Pathology and ‡Dermatology, the Finsen Institute, University of Copenhagen, Denmark

SUMMARY The reactivity of a monoclonal antibody against human T lymphotropic retrovirus (antibody 12/1-2, recognising the HTLV-1 p19 internal core viral protein) with benign and malignant cutaneous biopsy specimens was examined and compared with results obtained on normal skin, on various other human cells and tissues, and on immunoblotted extracts of tonsil squamous epithelium. In keeping with previous studies, 12/1-2 labelled a proportion of the thymic epithelial stroma and the entire layer of basal cells in stratified non-keratinised and keratinised epithelium. Furthermore, antibody 12/1-2 reacted with basal cell carcinomas and showed an essentially identical staining pattern in normal skin, cutaneous T cell lymphomas, and a range of benign dermatoses. The dot blot preparations showed that 12/1-2 recognised an antigen associated with keratin intermediate filaments. These data indicate that antibody 12/1-2 forms a useful marker for subsets of epithelial cells, which presumably participate in T cell education, and that a range of cutaneous disorders of widely different aetiology show no abnormalities in epithelial expression of this antigen.

In recent years the molecular characteristics of the exogenous human T cell leukaemia and lymphoma retrovirus (HTLV-1)¹ have been extensively investigated, and the amino acid and DNA sequences of the major HTLV-1 internal core viral proteins (p24, p19) are now known.²⁻⁴ Furthermore, monoclonal antibodies have been produced against both proteins.⁵⁻⁸

Whereas all of the former (anti-p24) reagents seem to be strictly HTLV-1 specific,⁷ several of the anti-p19 antibodies have shown reactivity with normal human cell types,⁸⁻¹⁰ including lymphocytes, brain, and a range of epithelial cells (antibody HTLV-2); kidney (antibody HTLV-4); and the neuroendocrine component of the thymic epithelial stroma and tonsil squamous epithelium (antibody 12/1-2). Furthermore, more recently the 12/1-2 antigen has also been detected in normal epidermis.¹¹ This finding has been of particular interest because the properties of T cell education have been ascribed not only to the thymic stroma,¹² but also to tonsil epithelium¹³ and epidermal cells.¹⁴

In this study 12/1-2 expression, in a range of benign and malignant cutaneous biopsy specimens, was investigated and compared with that of normal skin, various other human organs, and dot blot preparations of tonsil squamous epithelium extracts.

Material and methods

CELLS AND TISSUE

Skin punch biopsy specimens from benign or malign

Table 1 Details of cutaneous biopsy specimens studied

Disorders studied	Diagnosis	No of samples
Normal and benign biopsies	Normal	7
	Patch test biopsies	3
	Eczema	2
	Psoriasis	3
	Lichen planus	2
	Atopic dermatitis	1
Malignant lymphomas	Mycosis fungoides	10*
	Sezary syndrome	4
Solid tumours	Basal cell carcinoma	3
	Squamous cell carcinoma	3
	Malignant melanoma	4

*Comprised four patients with antibodies against HTLV-1, as determined by the use of an indirect ELISA assay.¹⁵

Table 2 Source and specificity of monoclonal antibodies

Antibody	Specificity	Source (and reference)
12/1-2	HTLV-1 p19; epithelial cells	Gallo RC ^{5 10 11}
Le61	40-45 kd keratins	Lane EB ¹⁸
KL1	55-57 kd keratins	Viac J ¹⁹
PK63	50-67 kd keratins	Pulford KAF
PK110	50-67 kd keratins	Pulford KAF
PK141	46-59 kd keratins	Pulford KAF
anti-HLA-DR	HLA-DR	Becton Dickinson
KB90	Macrophages (the p150,95 molecule)	Mason DY

nant cutaneous disorders (Table 1) were obtained from patients attending the dermatology department at the Finsen Institute. Normal skin and blood mononuclear cells were obtained from healthy adult volunteers. Samples from various other types of normal human cells and tissues, including bone marrow, thymus, spleen, lymph node, tonsil, lung, salivary gland, oesophagus, stomach, large intestine, liver, kidney, bladder, prostate, endometrium, thyroid, adrenal, pancreas, testis, and ovary were drawn from the frozen tissue banks of the departments of pathology at the John Radcliffe Hospital or the Finsen Institute. All the samples from the normal organs had been obtained either from fresh surgical excision specimens or at necropsy no later than six hours after death. In the cutaneous lymphomas the presence of serum antibodies against HTLV-1 was assessed by the use of an indirect enzyme linked immunosorbent assay described previously.¹⁵

PREPARATION OF CRYOSTAT SECTIONS

The tissue samples were embedded in OCT compound (Tissue-tek II, Miles Laboratories), frozen, and stored in liquid nitrogen. Cryostat sections (6 µm) were fixed in acetone, air dried overnight at room temperature, and either stained immediately or wrapped in aluminium foil and stored at -20°C until staining.¹⁶ After storing at -20°C the preparations were allowed to warm to room temperature before unwrapping. Cytospin preparations of Lymphoprep isolated blood and bone marrow cells were prepared as described previously.¹⁷

ANTIBODIES

Table 2 shows monoclonal antibodies used in this study. Immune complexes of alkaline phosphatase and mouse monoclonal antialkaline phosphatase (APAAP complexes) were prepared as described previously.¹⁷ Unconjugated rabbit antimouse immunoglobulin, peroxidase conjugated rabbit antimouse immunoglobulin, and peroxidase conjugated swine antirabbit immunoglobulin were purchased from Dakopatts, Copenhagen, Denmark.

IMMUNOENZYMATIC STAINING OF CRYOSTAT SECTIONS AND CYTOSPINS

Staining was performed by an immunoperoxidase method in three stages¹⁶ or by an alkaline phosphatase:antialkaline phosphatase (APAAP) technique.¹⁷ Endogeneous tissue alkaline phosphatase activity was inhibited by adding 1 mM levamisole (Sigma) to the substrate solution.²⁰

TISSUE EXTRACTION

Squamous epithelium was dissected out from fresh tonsil, taking care to include as little lymphoid tissue as possible. This tissue was minced, homogenised in 25 mM Tris hydrochloric acid (pH 7.4) at 4°C, and centrifuged for 10 minutes at 10 000 g, yielding a supernatant containing water soluble proteins.²¹ The pellet was resuspended and homogenised in 20 volumes of 25 mM Tris hydrochloric acid (pH 7.4) containing 1% Nonidet P40 (BDH), agitated at room temperature for 10 minutes, and centrifuged.¹⁸ This supernatant comprised the Nonidet P40 soluble fraction. The keratin proteins were extracted from the remaining pellet by boiling for five minutes with 10 volumes of the Tris buffer containing 10 mM dithiothreitol (Sigma) and 1% SDS.²¹ All solutions used contained the proteolytic inhibitors 2.5 mM iodoacetamide and 2 mM phenylmethylsulphonyl fluoride (Sigma).

DOT BLOT PREPARATIONS

Samples of the water soluble proteins, the Nonidet P40 fraction, and the keratin extract obtained after boiling were placed on to nitrocellulose paper (Schleicher-Schull, 0.45 µm pore size) in 8 µm aliquots. The paper was allowed to dry for 10 minutes at room temperature. The free protein binding sites of the paper were then blocked by incubation with 3% bovine serum albumin and phosphate buffered saline for 30 minutes at 37°C. The paper was subsequently incubated with antibody 12/1-2 or with control antibody KB90 (of irrelevant specificity, Table 2) for 10 minutes, washed, and stained with the APAAP method,¹⁷ using a modification of the method described by Leary *et al.*²² Briefly, 5 mg nitroblue tetrazolium salt (Sigma) dissolved in 13 ml 1 M diethanolamine (pH 9.8) at 37°C was added to 2 mg 5-bromo-4-chloro-3-indoyl-phosphate (Sigma) dissolved in 50 µl dimethylformamide. This solution was then poured on to the dot blot preparations. The reaction was allowed to proceed in the dark for five to 15 minutes and terminated by washing in tap water.

Results

IN SITU REACTIVITY OF ANTIBODY 12/1-2

Skin biopsy specimens

In normal skin antibody 12/1-2 labelled the cyto-

Fig. 1 12/1-2-positive basal cells in epidermis (a) and tonsil squamous epithelium (b). $\times 400$

plasm of basal keratinocytes, whereas the superficial epidermal layers were negative (Fig. 1). Essentially identical staining patterns were seen in a range of benign dermatoses (Table 1) and also in cutaneous T cell lymphomas. In particular, no differences were identified in normal compared with hyperplastic (psoriatic) epidermis.

Whereas 12/1-2 usually labelled the entire layer of basal cells, a focal basal cell staining pattern was observed in biopsy specimens from lichen planus, a condition associated with areas of destruction of the basal keratinocytes. A similar pattern was also found in some cutaneous T cell lymphoma cases in which the malignant cells showed a very pronounced tendency to infiltrate or destroy epidermal cells.

In accordance with the staining pattern found in normal epidermis, antibody 12/1-2 labelled basal cell carcinomas and was negative both with squamous cell carcinomas and malignant melanomas. None of the antikeratin antibodies showed a similar reactivity pattern, either in normal skin or in cutaneous epithelial tumours (Table 3).

In all skin biopsy specimens labelling with antibody 12/1-2 was confined to keratinocytes. In particular, the lymphocytes in both HTLV-1 positive and HTLV-1 negative cutaneous T cell lymphoma cases were 12/1-2-negative.

Thymus

As expected, antibody 12/1-2 reacted with dendritic cells in the thymic subcapsular and medullary regions and also stained scattered cells in the cortical area (Table 3 and Fig. 2). Serial sections stained with antibodies against HLA-DR, and keratins contained numerous cells of similar shape and tissue distribution, indicating that 12/1-2 reacted with the HLA-DR positive cells which constitute the thymic epithelial stroma. It was also evident, however (by comparing the reactions of 12/1-2 with those of anti-HLA-DR and antikeratin antibodies) that 12/1-2 was

Table 3 Proportion and distribution of 12/1-2-positive epithelial cells in skin and thymus compared with anti-keratin and HLA-DR antibodies

Antibody	Normal thymic epithelium				Normal epidermis		Cutaneous carcinomas	
	Subcapsular cells	Cortical cells	Medullary cells	Hassel's corpuscles	Basal cells	Cells above the basal layer	Basal cell	Squamous cell
12/1-2	+	+/-	+	-	+	-	+	-
Le61	-	+/-	+	-	-	-	-	-
KL1	+/-	+	+	+	-	+	+	+
PK63	-	+/-	-	+	+/-	+	-	+
PK110	-	-	-	+	-	+	-	+
PK141	-	+/-	+/-	+	-	+	-	+
anti-HLA-DR	+	+	+	+	-	-	-	-

+ = labelling of most of the cells.

+/- = labelling of a proportion of the cells.

- = negative.

Fig. 2 12/1-2-positive epithelial cells in subcapsular and medullary regions of thymus. Note also scattered 12/1-2-positive cortical cells and negative Hassall's corpuscles (arrow). $\times 150$.

expressed on only a proportion of the cortical stromal cells (Table 3). Furthermore, in contrast to the anti-keratin antibodies KL1, PK63, PK110 and PK141, 12/1-2 did not react with Hassall's corpuscles (Fig. 2).

Fig. 3 Dot blot preparation of water soluble proteins (a), Nonidet P40 soluble proteins (b), and boiled keratin extracts (c) from tonsil squamous epithelium. 12/1-2 reactivity is confined to keratin proteins present in (c).

Other human tissues

Immunostaining of various other types of human tissues showed that antibody 12/1-2 also labelled the cytoplasm of basal cells in stratified non-keratinised epithelium in tonsil (Fig. 1), oesophagus, and large salivary ducts, whereas all simple and glandular epithelia as well as all non-epithelial cells were negative.

Nitrocellulose dot blot preparations

Preparations of the water soluble proteins and the Nonidet P40 extract of the tonsil squamous epithelium gave negative or very weak reactions with 12/1-2, whereas the keratin proteins extracted from the detergent insoluble pellet by boiling gave reactions of moderate or strong intensity (Fig. 3). Control antibody KB90 (against the p150,95 molecule on macrophages) did not show any staining of the keratin proteins in the dot blot preparations.

Discussion

The results reported above extend other recent investigations⁸⁻¹¹ and indicate that monoclonal antibody 12/1-2 (against HTLV-1 p19 and its precursor protein)^{5,8} also reacts with normal human epithelial cells present in the thymic stroma and in the basal layers of stratified non-keratinised and keratinised epithelium. Furthermore, antibody 12/1-2 labels basal cell carcinomas and shows an essentially identical staining pattern in normal skin, benign dermatoses, and cutaneous T cell lymphomas.

Several explanations for the reactivity of antibody 12/1-2 with both HTLV-1 and normal human cells

have been considered in the past, including the possibility of a host origin of HTLV-1 p19.¹⁰ There is now definite evidence from amino acid and DNA sequence analysis, however, that HTLV-1 p19 is a gag region encoded viral protein.²⁻⁴ Furthermore, it has been shown that the normal 12/1-2 positive thymic cells do not show gene sequence homology with HTLV-1.¹⁰ While these studies have not precluded that expression of identical epitopes in HTLV-1 and normal epithelial cells may reflect homology between exogenous and endogenous retroviral DNA sequences,²³ they have provided conclusive evidence that antibody 12/1-2 reacts with both viral and host proteins.

Even though there are, as yet, no data available concerning the molecular characteristics of the 12/1-2 positive epithelial antigen, the DNA sequence studies mentioned above have strongly suggested that the 12/1-2 positive viral and host proteins are unlikely to be similar. This is supported by the finding in this investigation that the 12/1-2 positive epithelial antigen is present in conventionally prepared keratin extracts.¹⁸ It is not yet clear whether the determinant forms part of the filament core or may be present on an associated molecule, although there is indirect evidence from this and previous studies in favour of the second possibility. First, in thymic epithelial cells the epitope is exposed on cell surfaces.¹⁰ Second, unlike keratin,²⁴ the antigen detected by antibody 12/1-2 shows an essentially identical distribution in normal and hyperplastic (psoriatic) epidermis; and third, the staining pattern of 12/1-2 with normal and neoplastic epithelial cells differs considerably from that of a range of antibodies against different keratin classes.

The hypothesis that antibody 12/1-2 may detect a keratin associated component obviously does not preclude the possibility that "antigen masking" of keratins in tissue sections²⁵ may have contributed to the staining results obtained in this and previous studies.^{10,11} Nevertheless, in the intact organisms the 12/1-2 epitope is exposed in only certain types of epithelial cells (see above), and it has been proposed that the epitope may define related epithelial cells with common properties in T cell differentiation.¹¹ In keeping with this assumption is the finding that 12/1-2 positive epithelial cells contain thymopoietin,²⁶ express neuronal ganglioside,²⁷ and share a number of other phenotypic properties.^{11,28} Furthermore, several other independent observations have provided increasing evidence that epidermis may have a similar functional role in T cell differentiation to that of the thymic stroma.^{14,29,30}

The fact that HTLV-1 infected T lymphocytes share a cell surface membrane associated epitope³¹ with normal epithelial cells, which presumably participate in T cell education, also raises the intriguing possibility that immune reactions against the normal

cells may occur during HTLV-1 infection,⁷ and that these could result in impaired T cell maturation. Even though it has been well documented, however, that antibodies against the 12/1-2 epitope are often produced during HTLV-1 infection,³² their role in natural immune responses is as yet not fully understood.³¹ This aspect will require clarification in future investigations. This also applies to antibodies against other antigenic sites coexpressed by HTLV-1 p19 and normal human cells, such as those defined by antibodies HTLV-2 and HTLV-4.⁸

Our present study indicates that antibody 12/1-2 detects a keratin associated component and that it forms a useful marker for subpopulations of epithelial cells, which may share functional properties in T cell maturation. Neither benign dermatosis nor cutaneous T cell lymphomas show immunohistological abnormalities in epithelial expression of the antigen detected by antibody 12/1-2.

We thank Drs RC Gallo, P Sarin, and Kay Ulrich for valuable discussions during preparation of this work. The expert technical help of Lotte Laustsen is also acknowledged. Antibodies 12/1-2, KL1, and Le61 were kindly made available by Dr RC Gallo, J Brochier, and EB Lane, respectively. This study was supported by the Danish Cancer Society, the Leukaemia Research Fund, and the Wellcome Trust.

References

- Sarin PS, Gallo RC. Human T-lymphotropic retroviruses in adult T cell leukemia-lymphoma and acquired immune deficiency syndrome. *J Clin Immunol* 1984;4:415-23.
- Seiki M, Hattori S, Hirayama Y, Yoshida M. Human adult T-cell leukaemia virus: complete nucleotide sequence of the proviral genome integrated in leukaemia cell DNA. *Proc Natl Acad Sci USA* 1983;80:3618-22.
- Oroszlan S, Copeland TD, Kalyanaramen VS, Sarnagadharan MG, Schultz AM, Gallo RC. Chemical analysis of human T-cell leukaemia virus structural proteins. In: Gallo RC, Essex ME, Gross L, eds. *Human T-cell leukaemia/lymphoma virus*. USA: Cold Spring Harbor Laboratory, 1984:101-10.
- Hattori S, Kiyokawa T, Imagawa K-I, et al. Identification of gag end env gene products of human T-cell leukaemia virus (HTLV). *Virology* 1984;136:338-47.
- Robert-Guroff M, Ruscetti FW, Posner LE, Poiesz BJ, Gallo RC. Detection of the human T cell lymphoma virus p19 in cells of some patients with cutaneous T cell lymphoma and leukaemia using a monoclonal antibody. *J Exp Med* 1981;154:1957-64.
- Tanaka Y, Koyanagi Y, Chosa T, Yamamoto N, Hinuma Y. Monoclonal antibody reactive with both p28 and p19 of adult T-cell leukaemia virus specific polypeptides. *Gann* 1983;74:327-30.
- Palker TJ, Bolognesi DP, Haynes BF. Human T-cell leukaemia/lymphoma virus: studies on host-virus interaction. *Curr Top Microbiol Immunol* 1984;115:247-66.
- Palker TJ, Searce RM, Ho W, et al. Monoclonal antibodies reactive with human T cell lymphotropic virus (HTLV I) p19 internal core protein: cross-reactivity with normal tissues and differential reactivity with HTLV types I and II. *J Immunol* 1985;135:247-53.

- ⁹ Haynes BF, Palker TJ, Robert-Guroff M, *et al.* Monoclonal antibodies against human T-cell leukaemia/lymphoma virus p19 and p24 internal core proteins: spectrum of normal tissue reactivity and use as diagnostic probes. In: Gallo RC, Essex ME, Gross L, eds. *Human T-cell leukaemia/lymphoma virus*. USA: Cold Spring Harbor Laboratory, 1984:197-203.
- ¹⁰ Haynes BF, Robert-Guroff M, Metzgar RS, *et al.* Monoclonal antibody against human T cell leukaemia virus p19 defines a human thymic epithelial antigen acquired during ontogeny. *J Exp Med* 1983;157:907-20.
- ¹¹ Haynes BF. The human thymic microenvironment. *Adv Immunol* 1984;36:87-142.
- ¹² Cantor H, Weissman I. Development and function of subpopulations of thymocytes and T lymphocytes. *Prog Allergy* 1976;20:1-64.
- ¹³ Oosterom R, Kater L, Rademarkers LHPM. How unique is the thymus? Conditioned media from thymus and tonsil epithelial cultures share biological activities in T-cell maturation. *Clin Immunol Immunopathol* 1981;19:428-35.
- ¹⁴ Streilein JW. Skin-associated lymphoid tissue (SALT): origins and functions. *J Invest Dermatol* 1983;80:12s-16s.
- ¹⁵ Saxinger C, Gallo RC. Application of an indirect enzyme-linked immunosorbent assay microtest in the detection and surveillance of human T-cell leukaemia-lymphoma virus. *Lab Invest* 1983;49:371-7.
- ¹⁶ Stein H, Gerdes J, Schwab U, *et al.* Identification of Hodgkin and Sternberg-Reed cells as a unique cell type derived from a newly-detected small-cell population. *Int J Cancer* 1982;30:445-59.
- ¹⁷ Cordell JL, Falini B, Erber W, *et al.* Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219-29.
- ¹⁸ Lane EB. Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. *J Cell Biol* 1982;92:665-73.
- ¹⁹ Viac J, Reano A, Brochier J, Staquet M-J, Thivolet J. Reactivity pattern of a monoclonal antikeratin antibody (KL1). *J Invest Dermatol* 1983;81:351-4.
- ²⁰ Ponder BA, Wilkinson MM. Inhibition of endogeneous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. *J Histochem Cytochem* 1981;29:981-4.
- ²¹ Tseng SCG, Jarvinen MJ, Nelson WG, Huang J-W, Woodcock-Mitchell J, Sun T-T. Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. *Cell* 1982;30:361-72.
- ²² Leary JJ, Bligati DJ, Ward DC. Rapid and sensitive colorimetric method for visualizing biotin-labelled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose. *Proc Natl Acad Sci USA* 1983;80:4045-9.
- ²³ Suni J, Narvanen A, Wahlstrom T, Lehtovirta P, Vaheri A. Monoclonal antibody to human T-cell leukaemia virus p19 defines polypeptid antigen in human choriocarcinoma cells and syncytiotrophoblasts of first-trimester placentas. *Int J Cancer* 1984;33:293-8.
- ²⁴ Weiss RA, Guillet GYA, Freeberg IM, *et al.* The use of monoclonal antibody to keratin in human epidermal disease: alterations in immunohistochemical staining pattern. *J Invest Dermatol* 1983;81:224-30.
- ²⁵ Sun T-T, Eicher R, Nelson WG, *et al.* Keratin classes: molecular markers for different types of epithelial differentiation. *J Invest Dermatol* 1983;81:109-15.
- ²⁶ Chu AC, Patterson JAK, Goldstein G, Berger CL, Takezaki S, Edelson RL. Thymopoietin-like substance in human skin. *J Invest Dermatol* 1983;81:194-7.
- ²⁷ Haynes BF, Shimizu K, Eisenbarth GS. Identification of human and rodent thymic epithelium using tetanus toxin and monoclonal antibody A2B5. *J Clin Invest* 1983;71:9-14.
- ²⁸ Yonish-Rouach E, Schmitt D, Viac J, Knowles R, Cordier G, Brochier J. Monoclonal anti thymic cell antibodies detecting epidermal cells. *Thymus* 1984;6:67-78.
- ²⁹ Luger TA, Stadler BM, Luger BM, *et al.* Characteristics of an epidermal cell thymocyte-activating factor (ETAF) produced by human epidermal cells and a human squamous cell carcinoma cell line. *J Invest Dermatol* 1983;81:187-93.
- ³⁰ Rubinfeld MR, Silverstone AE, Knowles DM, *et al.* Induction of lymphocyte differentiation by epidermal cultures. *J Invest Dermatol* 1981;77:221-4.
- ³¹ Aoki T, Hamanda C, Ohno S, *et al.* Location of human T-cell leukaemia virus (HTLV) p19 antigen on virus-producing cells. *Int J Cancer* 1984;33:161-5.
- ³² Robert-Guroff M, Fahey KA, Maeda M, Nakao Y, Ito Y, Gallo RC. Identification of HTLV p19 specific natural human antibodies by competition with monoclonal antibody. *Virology* 1982;122:297-305.

Request for reprints to: Dr Elisabeth Ralfkiaer, Department of Pathology, The Finsen Institute, University of Copenhagen, 49 Strandboulevarden, DK-2100 Copenhagen Ø, Denmark.