RNA Purification from Epidermal Suction Blisters

METTE KRISTENSEN, CHRISTIAN GRØNHØJ LARSEN, POUL JØRGENSEN
and KIRSTEN PALUDAN

Department of Dermatology, University of Aarhus, Martselsborg Hospital and Department of Molecular Biology and Plant Physiology, University of Aarhus, Aarhus, Denmark

Certain inflammatory skin diseases are accompanied by increased cytokine concentrations in the epidermis. To determine whether these cytokines are synthesized in the epidermis or exported from underlying tissues, epidermal RNA was analysed for the presence of their messenger RNAs. We report a method for RNA extraction from pure epidermal samples isolated by the suction blister method. The yield of total RNA was sufficient for hybridization experiments (12–38 µg per seven blisters, 5 mm in diameter). Using RNA extracted by this method, we have demonstrated the presence of messenger RNA for glyceroldehyde-3-phosphate-dehydrogenase in 13 preparations from suction blisters obtained from tuberculin skin reactions, positive patch test reactions, or normal skin. We did not, however, observe messenger RNA for interleukin 1α or 8 in these preparations. Key words: Interleukin 1α; Interleukin 8.

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K. Paludan, Department of Dermatology, University of Aarhus, Martselsborg Hospital, DK-8000, Aarhus C, Denmark.

The expression and secretion of various cytokines from the skin are studied intensively in dermatological disorders. It is of utmost importance, however, to know which cells are participating, when cytokine expression is measured in skin samples, because cytokines are not equally expressed in various skin and inflammatory cells. Most studies are performed on skin biopsies where epidermal, dermal, and inflammatory cells such as monocytes are present. In vivo studies of cytokine messenger RNA (mRNA) expression in epidermal keratinocytes can therefore only be studied accurately using the suction blister technique (1, 2). We have previously observed that epidermal tissue homogenate contains increased levels of interleukin 1 activity and lymphocyte chemoattractant factor when exhibiting an allergic cell-mediated immune response (3, 4, 5) and we consequently wanted to elucidate the role of the keratinocyte in the production of these factors. Preparation of RNA from suction blisters has, however, hitherto been problematic due to the small amount of tissue available. This study presents our method for RNA extraction from epidermal suction blisters using a modification of the method of Chomezynski & Sacchi (6).

MATERIALS AND METHODS

Homogenization and RNA extraction

Suction blisters were generated as described elsewhere (1, 2). After removal of the suction cups, the seven epidermal suction blisters of 5 mm diameter each were placed in 1 ml of GTC buffer (4 M guanidinium thiocyanate, 25 mM sodium citrate pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) with 3.3 ml of 30% antifoam (Sigma), and immediately frozen in liquid nitrogen. Samples were homogenized while still partly frozen in order to liberate RNA into the protecting buffer. Homogenization was for 4 × 35 s with a Kinematica AG polytron PT 3000, dispersing tool PTDA 3007/2, with cooling on ice between runs. Fragments stuck in the dispersing tool were liberated and homogenized by a 15-s run in 1 ml of GTC buffer containing antifoam and pooled with the first homogenate. Total RNA extraction was performed by adding 200 µl 2 M sodium acetate pH 4.0, 2 ml water saturated phenol, and 400 µl chloroform-isoamylalcohol (49:1) to the 2 ml of epidermal homogenate. The samples were mixed vigorously and left on ice for 10 min. After centrifugation at 10,000 × g for 25 min at 4°C, RNA was precipitated from the aqueous phase with 2 ml isopropanol for 1 h at -20°C, collected by centrifugation at 10,000 × g for 25 min at 4°C, dissolved in 0.3 ml GTC, and reprecipitated with 0.3 ml isopropanol. The pellet was washed three times with 80% ethanol, air dried, and redissolved in RNAase-free water.

Northern blotting and hybridization

Samples were denatured, electrophoresed through 1% denaturing agarose gels, and transferred to GeneScreen membranes (New England Nuclear) in 20 × SSC (3 M NaCl, 0.3 M Na citrate, pH 7.0). Radioactive probes were prepared by random oligonucleotide priming (7) of restriction fragments purified from agarose gels. The IL-1α cDNA was contained in a 1.7 kb EcoRI–HindIII fragment cloned in pUC19, the IL-8 cDNA in a 1.5 kb EcoRI frag-
Fig. 1. UV light absorption spectrum of suction blister RNA sample. A 150-fold dilution of a sample was scanned at 220–320 nm in a Shimadzu spectrophotometer.

The GAPDH cDNA from the pBR322 fragment, cloned in pUC9, was used to derive from pBGAPDH-13 (Fig. 2). Hybridizations were performed at 65°C with a probe concentration corresponding to approximately 10⁶ cpm/ml in 4 x SSC, 1% SDS (sodium dodecyl sulphate), 5 x Denhardt’s reagent (0.1% polyvinylpyrrolidone, 0.1% Ficoll, 0.1% bovine serum albumin), and 200 µg/ml sheared, denatured E. coli DNA. Washing conditions were 0.2 x SSC, 0.5% SDS at 65°C.

RESULTS

We routinely used suction cups with 4 holes of 5 mm in diameter for in vivo studies. The total size of epidermis is then 1.4 cm².

In 13 experiments we obtained from 12 to 38 µg RNA with an average of 22 µg. The 260 nm/280 nm UV light absorption ratio was 1.8 indicating a high degree of RNA purity (Fig. 1). RNA integrity was confirmed by gel electrophoresis (Fig. 2).

Since suction blisters are small and resilient, their efficient homogenization constitutes a major problem of RNA extraction. We found that the smallest polytron dispersing tool available gave the best results, and that this method was preferable to grinding. To avoid RNA degradation upon thawing, it is essential that the tissue is homogenized while only partially thawed. Omission of this may result in reduced yields.

Northern blots were prepared of RNA from suction blisters taken 1, 2, 3, and 24 h after tuberculosis injection (1 person), 4 h after tuberculosis injection (2 persons), and 4 h (1 person) or 16 h (2 persons) after application of patch tests. All test persons had previ

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ously shown positive reactions, but at the time of testing the reactions were either negative or starting to show a positive reaction. We also prepared RNA from suction blisters taken from normal skin (5 persons).

Blots were hybridized sequentially to cDNA probes for IL-1α, IL-8, and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a housekeeping enzyme present in low amounts in all cells (9). Integrity of mRNA was confirmed by the GAPDH probe detecting a faint, but distinct band (Fig. 3, lanes 1–3), but even after over-exposure we detected no IL-1 or IL-8 signal (data not shown), although these probes gave clear signals with RNA from cultured cells (fibroblasts, endothelial cells, keratinocytes, monocytes) treated with bacterial lipopolysaccharide (LPS). Having excluded trivial reasons for the failure to detect IL-1α and IL-8 messenger RNA in suction blister samples from tuberculin and patch test reactions, we conclude that the IL-1α and IL-8 messengers are below the detection level of the assay or not expressed at the chosen time points.

**DISCUSSION**

We have shown that small and clinically relevant amounts of pure epidermis, isolated by the suction blister technique, are sufficient for obtaining enough total RNA to perform studies on messenger RNA expressions.

Similar observations have been reported. In a separate study, using cultured human cells, we have shown that IL-1α-stimulated keratinocytes synthesize substantially less IL-8 mRNA than dermal fibroblasts, monocytes, or endothelial cells under identical conditions (10). Therefore, when studying cytokine expression in the skin, it is very important to determine which cells are present in the tissue sample.

Since cytokines can be biologically active in concentrations as low as $10^{-15}$ M, mRNA amounts below the detection limit of the assay may be sufficient for synthesis of IL-1 and IL-8 to concentrations capable of the biological activity observed in inflammatory skin reactions. Also, the possibility remains that epidermal cytokines may be imported from other tissues. Further time course studies are needed in the search for the possibly transient presence of epidermal IL-1 and IL-8 mRNAs.

In one patient we have observed that epidermal suction blisters stimulated for 4 h with LPS do express IL-8 mRNA, and we have recently found that IL-8 mRNA can be found in normal unstimulated human epidermis using the polymerase chain reaction technique (data not shown).

In conclusion, the method reported here yields suction blister derived RNA of sufficient quality and quantity for Northern blot analysis for specific mRNAs, as shown by our detection of GAPDH mRNA in all samples. The method should therefore be useful for studies on expression in human epidermis of any peptide-mediator, enzyme, or structural protein for which the corresponding cDNA is available.

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Reactivity of HMB-45 Monoclonal Antibody with Sweat-gland Tumours of the Skin

JEAN KANITAKIS, CLAUDE HERMIER, BRIGITTE CHOUVET and JEAN THIVOLET

Laboratory of Dermatopathology/Inserm U209, Department of Dermatology, Ed. Herriot Hospital, Lyon, France

HMB-45, a monoclonal antibody claimed to be specific for malignant melanoma, has been observed to react with normal eccrine sweat glands and occasionally with normal mammary and bronchial epithelium. In this study we show that HMB-45 also decorates cells in approximately 15% of various sweat-gland tumours of the skin. This finding, along with the reported reactivity on mammary carcinomas further outlines the lack of absolute specificity of HMB-45 for cells of the melanocytic lineage.

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J. Kanitakis, Laboratory of Dermatopathology/Inserm U209, Department of Dermatology, Ed. Herriot Hospital, F-69437 Lyon Cedex 03, France.

HMB-45 is a monoclonal antibody raised against an axillary lymph node containing metastatic melanoma cells (1). This antibody was shown to react with fetal, neonatal and activated (but not normal) adult melanocytes, as well as junctional and dysplastic (but not benign intradermal) naevus cells; furthermore, HMB-45 decorates primary and metastatic malignant melanomas (MM), except those consisting of spindle-shaped cells (2–10). Although the antigen recognized by HMB-45 remains unknown, the possibility of using HMB-45 on routinely-fixed tissue sections has led to its widespread use in the immunohistochemical diagnosis of MM. However, despite early claims for absolute specificity of HMB-45 for cells of the melanocytic lineage, more recent articles have reported HMB-45 positivity in rare cases of breast carcinomas (5,11), plasmacytomas (5) and probable adenocarcinomas (12).

During the routine use of HMB-45 we have noticed, in keeping with previous reports (2, 3), that HMB-45 may also react with a cytoplasmic antigen of cells of the secretory coil of eccrine sweat-glands (SG). The present study was undertaken in order to further investigate the possible reactivity of HMB-45 with benign and malignant tumours of the skin originating from SG.

MATERIAL AND METHODS

Forty-seven cases of a variety of benign and malignant SG tumours (Table 1) were retrieved from the files of the Dermatopathology Laboratory of our Department of Dermatology. Specimens of normal skin and miscellaneous lesions were studied as controls. All tissue specimens had been conventionally fixed in 10% formalin and embedded in paraffin wax. 3-μm-thick sections were immunostained by applying an avidin-biotin-alkaline phosphatase technique (kit Vectastain, Vector, Burlingame, Calif.) as detailed elsewhere (13) without prior proteolytic treatment. HMB-45 antibody (purchased from Enzo Diagnostics, New York, NY) was used at a 1:400 dilution.

Negative controls comprised sections incubated with non-immune serum (instead of HMB-45); these proved consistently unlabelled.

RESULTS

On normal human skin and the various inflammatory lesions studied, HMB-45 antibody regularly and strongly decorated melanocytes of the hair bulb; melanocytes of the upper part of the hair follicle and of the epidermis were more rarely and less intensely labelled, especially in areas adjacent to inflammatory lesions. The labelling pattern was cytoplasmic and diffuse. In almost all the specimens studied,