Acta Derm Venereol (Stockh) 1991; 71: 423–457

## SHORT REPORTS

# RNA Purification from Epidermal Suction Blisters

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**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 glyceraldehyde-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.***

(Accepted February 4, 1991.)

Acta Derm Venereol (Stockh) 1991; 71: 423–426.

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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 chemotactic 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 Chomczynski & 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/l 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 $4 \times 15$ s with a Kinematica AG polytron PT 3000, dispersing tool PT-DA 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 $10,000 \times g$ 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 $10,000 \times g$ 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 RNase-free water.

### *Northern blotting and hybridization*

Samples were denatured, electrophoresed through 1% denaturing agarose gels, and transferred to GeneScreen membranes (New England Nuclear) in $20 \times$ 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 *Eco*RI–*Hin*dIII fragment cloned in pUC19, the IL-8 cDNA in a 1.5 kb *Eco*RI frag-

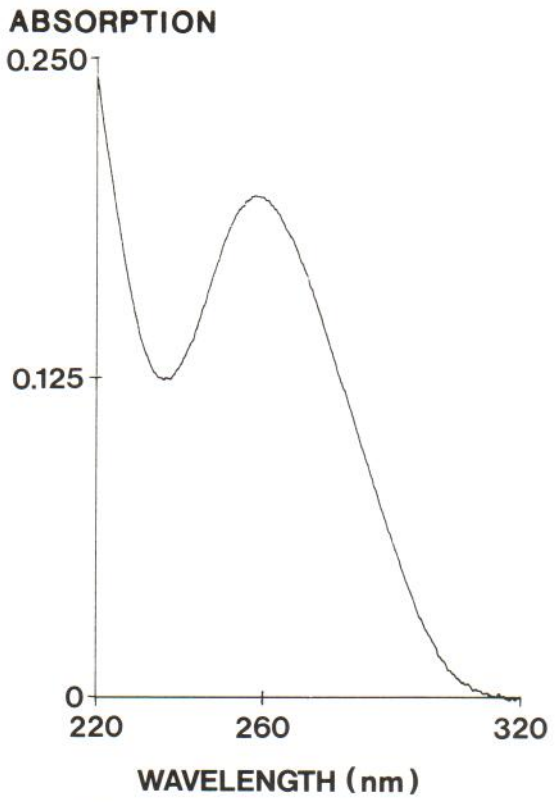


*Fig. 1.* UV light absorption spectrum of suction blister RNA sample. A 150-fold dilution of a sample was scanned at 220–320 nm in a Shimadzu spectrophotometer.

ment cloned in pBR322, and the GAPDH cDNA in a 1.3 kb *Pst*I fragment, subcloned in pUC9, originally derived from pRGAPDH-13 (8). Hybridizations were performed at 65°C with a probe concentration corresponding to approximately $10^6$ cpm/ml in 4 × SSC, 1% SDS (sodium dodecyl sulphate), 5 × 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 × SSC, 0.5% SDS at 65°C.

## RESULTS

We routinely used suction cups with 7 holes of 5 mm in diameter for in vivo studies. The total size of epidermis is then 1.4 $cm^2$.

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

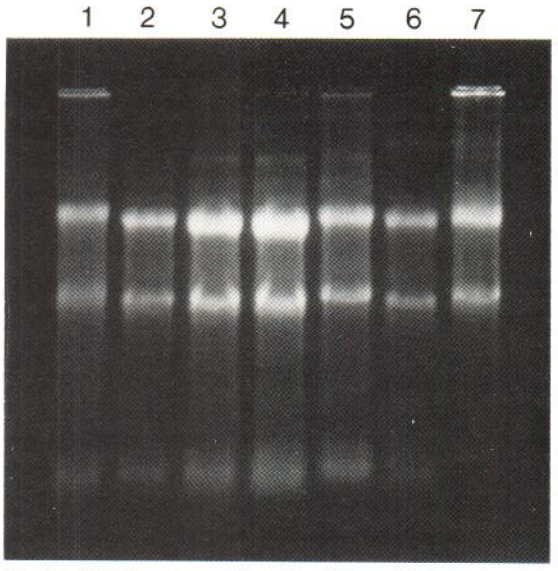


*Fig. 2.* Ethidium bromide-stained formaldehyde–agarose gel. *Lanes 1–5,* RNA from suction blisters (6 μg, 6 μg, 13 μg, 15 μg, and 13 μg, respectively); *lane 6,* monocyte RNA (5 μg); *lane 7,* AMA cell RNA (20 μg). In lanes 1 and 7, small amounts of contaminating DNA are seen, retained in the slots. The prominent bands are 28S, 18S, and 5S ribosomal RNA.

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 tuberculin injection (1 person), 4 h after tuberculin injection (2 persons), and 4 h (1 person) or 16 h (2 persons) after application of patch tests. All test persons had previ-

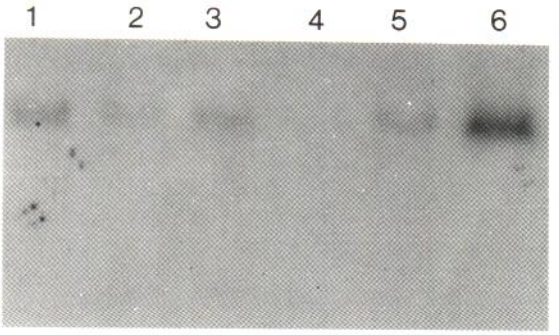


*Fig. 3.* Autoradiogram of Northern blot after hybridization to GAPDH probe. *Lanes 1–3,* suction blister RNA (29, 24, and 21 μg); *lanes 4* and *5,* monocyte RNA (2 and 20 μg); *lane 6,* AMA cell RNA (20 μg).

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 household 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 to our knowledge not previously 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.

## ACKNOWLEDGEMENTS

This work was supported by Institute for Experimental Clinical Research, University of Aarhus, the Danish Psoriasis Research Foundation, and the Danish Biotechnology Programme. We thank Dr Kouji Matsushima for providing the IL-1α and IL-8 cDNA clones, Dr Michael Etzerodt for technical advice and Professor Kristian Thestrup-Pedersen for helpful discussions.

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