Vol. 180, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1991 Pages 204-208

CELLULAR RETINOIC ACID-BINDING PROTEIN TYPE 2 mRNA IS OVEREXPRESSED IN HUMAN PSORIATIC SKIN AS SHOWN BY IN SITU HYBRIDIZATION

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Received August 27, 1991

Summary : In situ hybridization with full length mouse cellular retinoic acid-binding protein type 1 and cellular retinoic acid-binding protein type 2 cDNA derived RNA probes showed overexpression of cellular retinoic acid-binding protein type 2 mRNA in lesional hyperplastic psoriatic skin whereas cellular retinoic acid-binding protein type 1 mRNA was undetectable. This suggests that the previously reported increase of cellular retinoic acid-binding protein in psoriatic epidermis corresponds to increased translation of cellular retinoic acid-binding protein type 2 gene. Cellular retinoic acid-binding protein types 1 and 2 mRNAs were not detectable in normal epidermis; however, type 2 message was detected in non hyperplastic, non lesional skin of psoriatic patients thus before altered epidermal differentiation and hyperplasia are morphologically detectable.

Cellular retinoic acid-binding protein (CRABP) is thought to be involved in the mode of action of retinoids (1). Recently, a second type of CRABP, CRABP2, was detected by biochemical methods in neonatal mouse skin (2,3) ; Giguère et al (4) further identified by molecular cloning a CRABP2 mRNA in mouse embryo and in adult mouse skin.

Psoriasis is a common skin disease characterized by a non neoplastic hyperproliferative state of the epidermis which shows morphological and biochemical signs of alterd terminal differentiation. Using radio ligang binding assay, we have previously found an increase of CRABP in lesional skin as compared to non lesional skin in psoriatic patients and to the skin of non-psoriatic subjects (5,6). This assay did not allow to distinguish between CRABP1 and CRABP2. We now report overexpression of CRABP2 mRNA in psoriatic skin.

MATERIAL AND METHODS

Skin specimens : Six normal skin specimens were obtained from our local dermatosurgery staff. Nine biopsies from lesional and five from non lesional psoriatic skin were obtained after patient informed consent.

ABBREVIATIONS :
CRABP, cellular retinoic acid-binding protein; PAGE, polyacrylamide gel electrophoresis; SSC, standard sodium citrate.

0006-291X/91 $1.50

All specimens were quickly immersed in cold 2-methylbutane then in liquid nitrogen and kept at -70°C.
Four μm set cryostat skin sections were mounted on poly-l-lysine-coated slides, fixed for 1 min in 4% paraformaldehyde in PBS, rinsed for 1 min in cold PBS then kept in 70% ethanol at +4°C.

Probes synthesis : Mouse CRABP1 and mouse CRABP2 cDNAs were kindly provided by Prof. P.Chambon (CNRS-LGME, INSERM U-184, Strasbourg, France).
Full length 760 bp mouse CRABP1 cDNA (7,8) was subcloned into pGEM-4Z vector in EcoR1 site, cRNA anti-sense probe was transcribed using T7 RNA polymerase from Nde1 linear vector and control cRNA sense probe was transcribed using SP6 RNA polymerase from Sma1 linear vector. Full length 830 bp mouse CRABP2 cDNA (4) was cloned between EcoR1 and Hind3 sites of Bluescript SK+ vector, cRNA anti-sense probe was transcribed using T7 RNA polymerase from EcoR1 linear vector and control cRNA sense probe was transcribed using T3 RNA polymerase from Kpn1 linear vector. Both anti-sense probes hybridized transcripts of 1.1 kb and 1.3 kb respectively on Northern of total RNA from normal human skin and lesional psoriatic skin.
For in situ hybridization, transcriptions were carried out using a RNA labeling kit with digoxigenin-UTP (Boehringer Mannheim GmbH; Mannheim, Germany) according the manufacturer's protocole. With 1μg linear vectors as templates, > 5μg of digoxigenin-labeled RNAs were usually synthesized after 2 h of incubation at 37°C. The transcribed digoxigenin-labeled RNAs were shown to have the same length as the inserted cDNA by Northern blots using the Boehringer Mannheim's digoxigenin nucleic acid detection kit. Before use, the probes were subjected to partial alkakine hydrolysis to obtain RNA fragments approximately 250 nucleotides in length (9).

Prehybridization and hybridization : Slides were processed according to Springer et al. (10) with slight modifications as follows : after acetylation and glycine treatments, the hydrated sections, either pretreated with RNAse (5μg/ml) or not, were covered with 50μl of hybridization buffer containing 10ng of either anti-sense or sense probes. The sections were covered with coverslips for 4 h incubation at 50°C in a closed humid box. After washing, the sections were treated with RNAse (5μg/ml) for 30 min at 37°C, washed and left 2 h in 2X SSC containing 0.05% Triton X-100 and 2% normal sheep serum. After washing, the slides were incubated overnight at 4°C with the anti-digoxigenin alkaline phosphatase-conjugated antibody diluted 1/1000 in 100mM Tris-HCl (pH 7.5)/150mM NaCl containing 0.3% Triton X-100 and 1% normal sheep serum. For detection of the signal, slides were incubated in the dark for 3 h in the enzyme substrate, rinsed in 10mM Tris-HCl (pH 8.1) and 1mM EDTA (pH 8) then coverslipped using an aqueous mounting medium (Fluoprep ; Biomérieux, Marcy-l'Etoile, France). For all experiments, two control slides incubated with hybridization mix without probe were carried out at the same time in order to determine the alcaline phosphatase background.

RESULTS

No CRABP1 mRNA expresssion was found in any normal human skin specimens studied whereas a strong signal was detected in normal adult mouse epidermis (not shown). In involved psoriatic skin, a faint signal was observed in two out of nine cases (Table 1). It was localised in the deepest epidermal cell layers. No significant CRABP1 mRNA signal was detected in the upper dermis.

CRABP2 mRNA was not detected in normal human epidermis nor superficial dermis (Fig 1A). In contrast, specific signal for CRABP2 mRNA was found in psoriatic skin specimens (Fig 1B-C). The signal was both stronger and more constantly detected in lesional as compared to non lesional skin specimens of psoriatic patients (Table 1).

In lesional skin, CRABP2 mRNA was found in the epidermis and superficial dermis : in the epidermis the distribution was throughout all cell layers except the parakeratotic

Table 1. Skin in situ hybridization for CRABP1 and 2 mRNAs

	NORMAL SUBJECTS	PSORIATIC PATIENTS	
		non lesional	lesional
	n=6	n=5	n=9
CRABP1	- (6)	- (5)	- (7)
			± (1)
			+ (1)
CRABP2	- (6)	- (1)	- (0)
		+ (3)	+ (4)
		++ (1)	++ (4)
			+++ (1)
Radio ligand binding assay for CRABP (pmol/mg protein)*			
	3.8±0.9	3.3±1.5	30.3±6.2

- : no expression to +++ strong expression. Numbers in brakets indicate the number of specimens (patients) showing the pattern.

* data from references 11 and 12 (see text).

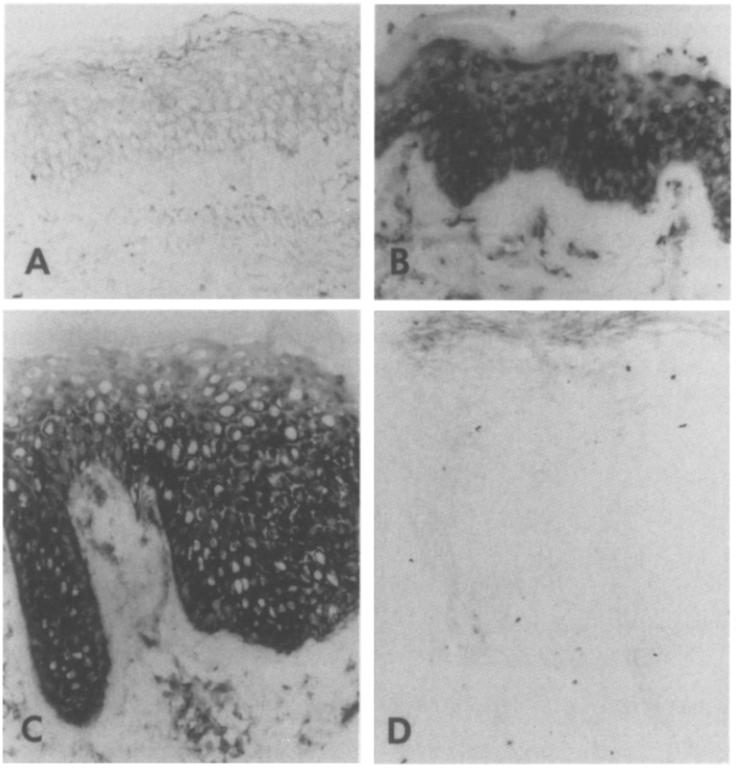

Figure 1. In situ hybridization with cRNA CRABP2 probes (see methods).
A : anti-sense probe on normal human skin specimen ; B : anti-sense probe on non hyperplastic, non lesional skin specimen from a psoriatic patient ; C : anti-sense probe on hyperplastic lesional psoriatic skin specimen ; D : sense probe on hyperplastic lesional psoriatic skin specimen.

horny layer (i.e. all living cells) in 7/9 cases and predominantly in basal and suprabasal cells in 2/9 cases (Fig 1C). In the superficial dermis, the signal was localized in cells which distribution might correspond to endothelial and peri-endothelial cells, some with spindle and dendritic shape (Fig 1C).

In the non lesional skin of psoriatic patients the CRABP2 mRNA signal was localised in both epidermis and superficial dermis but fewer cells were positive ; Fig 1B shows the strongest signal found in these series.

Specificity was demonstrated by clean negative control sections hybridized with sense probes (Fig 1D) and RNAse pretreated sections (not shown).

DISCUSSION

When radio ligand binding assay had been used for the detection of CRABP in keratomized biopsies of normal human skin (which includes epidermis and superficial dermis) about 3 pmol/mg protein was measured (11). Neither CRABP1 nor CRABP2 mRNAs could be detected in such specimens of normal human skin by in situ hybridization. This may indicate either a low sensitivity of the method and/or low transcription rates.

In keratomized biopsies of *lesional* psoriatic skin we previously found 30 pmol/mg protein of CRABP by radio ligand assay, i.e., 10 fold the value of normal skin (12). The present study provides two interesting observations on this line :

(i) a preferential increase of CRABP2 mRNA, that was strongly overexpressed, whereas CRABP1 mRNA was barely detectable. This indicates that the increase of CRABP that we previously detected by radio ligand assay (5,6,12) most probably corresponds to CRABP2 rather than to CRABP1. Recent observations from our laboratory (G. Siegenthaler, in preparation) with PAGE radio ligand assay indicate that CRABP2 is indeed overexpressed in psoriatic plaques. Psoriasis is a non neoplastic hyperplastic state of the epidermis which shows altered terminal differentiation. That CRABP2 rather than CRABP1 is overexpressed therein may help elucidating the modulation of the two forms of CRABP. The rate of formation of retinoic acid from retinol is increased in psoriatic lesions (13) ; this could lead to an increased concentration of this ligand in psoriatic cells thus increasing transcription of CRABP2 gene which may have a retinoic acid response element.

(ii) CRABP2 mRNA was also detected in cells localised beneath the epithelium in the superficial dermis. This suggests that part of the CRABP measured in keratomized biopsies by radio ligand methods (5,6,12) should originate from these cells. Further, the role of such cells (some of which might be dendrocytes as extrapolated from their dendritic shape), in the metabolism of retinoids in the skin is worth considering ; these cells may play a role in the constitution of the psoriatic lesion (14).

Although *non lesional* skin of psoriatic patients had not been found to express more CRABP binding activity than the skin of normal subjects (see Table 1), the present study identified increased CRABP2 mRNA in the former. This is of interest because the epidermis, where increased CRABP2 mRNA was found, did not show signs of either

hyperplasia or altered differentiation (see Fig 1B). This indicates that increased mRNA and thus possibly transcription of the CRABP2 gene occurs before these morphological changes are constituted. Overexpression of CRABP1 has recently been shown to reduce the differentiating effect of retinoic acid in F9 teratocarcinoma cells (15) ; whether the development of a psoriatic lesion may result from translation of the already overexpressed CRABP2 mRNA, thus leading to a reduced differentiating signal of endogenous retinoic acid, should await further studies.

ACKNOWLEDGMENTS

We acknowledge D. Gumowsky-Sunek for performing the skin biopsies, D. Grand for skillful technical assistance, G.Siegenthaler and C. Ucla for discussion and advices. Prof. P. Chambon is gratefully acknowledged for reading the manuscript. Supported in part by a grant from FNSRS 3200-009503.

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