Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues

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Summary

This paper reports further study of the identity and function of a protein shown to be elevated in serum from cystic fibrosis (CF) patients and clinically normal heterozygotes. Monoclonal antibodies, specifically recognizing the tentatively named cystic fibrosis antigen (CFAg), were produced. Immunoaffinity purification of CFAg from several sources revealed two components: $11 \times 10^3$ and $14 \times 10^3 M_r$ proteins. cDNA clones corresponding to each protein have been isolated. Data-base comparisons of the deduced amino acid sequences suggest that both genes encode related but distinct calcium-binding proteins. We propose the name calgranulin A and B, for the $11 \times 10^3$ and $14 \times 10^3 M_r$ components, respectively. It is clear from the assignment of the calgranulin genes to chromosome 1 that neither is the product of the mutant CF gene, which maps to chromosome 7.

We have used the monoclonal antibodies to study the tissue distribution of the two proteins in a wide-ranging immunohistological survey. Where possible the pattern of expression was confirmed by RNA blot analysis. Strong calgranulin expression in granulocytes was confirmed. In addition to myeloid cells, a restricted subset of normal stratified squamous epithelia were found to be calgranulin-positive. These included tongue, oesophagus and buccal cells, the last of which has been shown to have altered calmodulin activity in CF patients. Using indirect alkaline phosphatase staining, tissue sections of lung, pancreas and skin (normally considered sites where the CF defect is expressed) were not calgranulin-positive. However, by indirect immunofluorescence, nasal polyp sections showed weak patchy calgranulin expression in some epithelial cells, and stronger, higher frequency expression when such cells were briefly cultured.

A number of hyperproliferative, neoplastic or frankly malignant epithelia were found to express the two proteins. Calgranulin expression is a good marker for 'reactive' epithelium in skin and for squamous cell carcinomas of skin, lung and buccal tissues. The calgranulin-positive permanent cell line from a buccal squamous cell carcinoma may prove a suitable tool for unravelling the calgranulin-CF relationship.

Key words: cystic fibrosis, calcium-binding proteins, immunohistochemistry.

Introduction

Quantitative measurement of the serum levels of cystic fibrosis antigen (CFAg) was first made possible by the rocket immuno-assay of Manson & Brock (1980), although the presence of an abnormal protein on serum isoelectric focusing was first described by Wilson et al. (1975). CFAg levels were shown to fall into three genotype-dependent classes (Bullock et al. 1982). This pattern, particularly the intermediate levels in clinically unaffected heterozygotes, suggests that the increase of this protein in serum is closely related to the basic defect in this autosomal recessive disease, and that CFAg might be the product of the CF gene itself. By constructing mouse-human somatic cell hybrids, using parental cells of myeloid origin that express this differentiated cell product, we showed that CFAg is encoded by gene(s) on chromosome 1 (van Heyningen et al. 1985). When the assignment of the disease locus to chromosome 7 was announced (Knowlton et al. 1985; White et al. 1985; Wainwright et al. 1985), it was immediately clear that CFAg is not the product of the CF gene. However, in recessive conditions, knowing the identity and understanding the function of a secondary abnormality that is expressed in a gene-dose-dependent manner, and therefore likely to be caused by the underlying disease-causing
mutation, may reveal the nature of the basic defect. We therefore continued our efforts to determine the identity of CFAg.

With the aid of specific monoclonal antibodies (Hayward et al. 1986) the serum component was purified by immunoaffinity chromatography. SDS-PAGE analysis of the purified protein revealed two components of \(11 \times 10^3\) and \(14 \times 10^3 M_r\), but a unique \(N\)-terminal amino acid sequence was obtained (M. Novak, personal communication). A cDNA clone corresponding to this sequence was picked from a myeloid cDNA library and shown to possess strong homology with a family of intracellular calcium-binding proteins (Dorin et al. 1987). Recently, Odink et al. (1987) used a monoclonal antibody directed to macrophage migration inhibitory factor to isolate two low molecular weight protein components. Amino acid sequence data were obtained for each component (the \(N\) terminus of the larger 14K \((K = 10^3 M_r)\) protein was blocked) and the corresponding cDNA clones were isolated. Sequence analysis of the clone for the smaller protein showed it to be the same calcium-binding protein that we had isolated as CFAg. The cDNA for the 14K component was found to encode a second calcium-binding protein with a high degree of similarity to CFAg and to other members of the same family. Using the published sequence we isolated the corresponding clone from the myeloid cDNA library. It has been suggested (Andersson et al. 1988) that these same two proteins may be components of the leucocyte LI complex. To reduce confusion and in keeping with the naming of other members of the family, e.g. calcecin (Ferrari et al. 1987) and calbindin-D (Lee et al. 1987), we suggest the names calgranulin A and B for the 11K and 14K proteins, respectively.

In considering the involvement of the calgranulins in the aetiology of CF, it is necessary to ask whether these proteins are expressed in the tissues where the chloride channel abnormality has been demonstrated. A converse approach would be to look for disease-related abnormalities in calgranulin-positive cells, especially in granulocytes, which are the most likely source of the raised serum levels of these proteins. In this paper we explore the tissue distribution of the two calcium-binding proteins, using the monoclonal antibodies for immunohistochemical and protein blot analysis, and the cDNA probes for confirmatory RNA blot analysis.

Antibodies

CF557 and CF145, the mouse monoclonal antibodies directed against CFAg were used. These have been described (Hayward et al. 1986). A monoclonal antibody (AFP) against alphafetoprotein was used as negative control.

Staining and photography of tissue sections

Sections were incubated in a moist chamber at room temperature with neat hybridoma culture supernatant for 1h. They were then washed three times with PBS–BSA (1%, w/v, bovine serum albumin in Dulbecco's A phosphate-buffered saline) before incubation under the same conditions with alkaline phosphatase-conjugated (at 1:200 dilution) or FITC-conjugated (at 1:40 dilution) F(ab')_2 portion of a sheep antibody to mouse immunoglobulin G (Sigma).

For the alkaline phosphatase colour reaction the substrate was naphthol AS-B1 phosphate, sodium salt (Sigma), which, after phosphate hydrolysis, complexes with Fast Red TR salt to produce a water-insoluble red precipitate; endogenous tissue alkaline phosphatase activity, other than intestinal, was inhibited by the addition of 1 mmm-levamisole to the substrate mixture (Ponder & Wilkinson, 1981). Sections were counterstained with Mayer's haemalum and mounted in aqueous mountant. Sections were examined on a Leitz Ortholux microscope. Black-and-white photographs were taken on Pan X film using a Balzers B40-517.7 filter.

In fluorescence studies on fixed blood smears the nuclei were visualized by post-staining with the DNA intercalating dye Hoechst 33258, the emission from which can be distinguished from FITC fluorescence.

Fluorescent samples were mounted in PBS/glycerol and examined with a Leitz Ortholux II microscope using epillumination. Photographs were taken on Kodak TriX film.

Primary cell culture from nasal polyp tissue

Simple mucous nasal polyps surgically removed, predominantly from patients with atopic disease, were kindly provided by Dr Janet Wilson. Small samples were removed for direct examination following fixation in formal–saline. The remainder was finely minced and subjected to enzymic cell dispersal and in vitro culture, without feeder cells, in serum-free defined medium (F10 nutrient mixture containing: 2 mM-glutamine, 5 \(\mu\)g/mL transferrin, 5 ng/mL sodium selenite, 13 nM-1 epidermal growth factor, 5 ng/mL endothelial cell growth supplement, 10 ng/mL cholina toxin, 100 nm-hydrocortisone, 3 nm-triiodothyronine) as described by Yankaskas et al. (1985). RNA was prepared from one fresh whole polyp.

Cell culture and xenografting

TRI46, a cell line derived from a metastatic human buccal squamous cell carcinoma (Rupniak et al. 1985) was a gift from Dr Birgit Lane. It was grown in vitro in RPMI 1640 medium supplemented with 5% foetal calf serum. It was also propagated as xenograft tumours in immunosuppressed CBA mice (Busuttil et al. 1986). Tumours removed from successful grafts were used for the preparation of tissue extracts for protein blotting, for RNA isolation or fixed in formal–saline for histological analysis.

Protein blot analysis

Tissue extracts were prepared by sonication in PBS. Samples (20 \(\mu\)g) were prepared for electrophoresis by boiling in the presence of 1% (w/v) SDS and 2-5% (v/v) mercaptoethanol. "Rainbow" molecular weight markers (Amersham) were used. Samples were run in 12% polyacrylamide gels in 0-1 M-Tris–glycine, 0-2% SDS buffer, pH 9-4. Separated proteins

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were transferred to Hybond-C membranes (Amersham) by
electroblotting. Vacant membrane sites were blocked by wash-
ning the filters with Tris-buffered saline (TBS; 0.05 M-
Tris·HCl, 0.15 M-sodium chloride, pH 7.9) containing 3% (w/v) dried skimmed milk powder. Filters were then treated
with CF557, CF145 or negative control AFP hybridoma culture
supernatants for 1 h, washed, and exposed to 125I-labelled
staphylococcal protein A to identify the position of protein
bands to which the monoclonal antibodies bind. After repeated
washing with TBS–0.05% Tween 20 to minimize background
binding, the filters were dried and autoradiographed, using
preflashed Agfa Curix film.

RNA blot analysis
RNA was prepared from fresh or frozen tissue or from cultured
cell pellets by the guanidine hydrochloride method (Chirgwin
et al. 1979). A 10 µg sample of denatured total RNA per sample
was loaded onto 1-2% agarose gels prepared in 10 mM-sodium
phosphate (pH 6.5) buffer containing 18% formamide, electro-
phoresed overnight at 20 V cm⁻¹ and transferred to Hybond N
filters (Amersham). The filters were probed successively with
the cDNA probes: (1) 4B-7 for the 14K calgranulin B molecule,
isoalted and sequenced for verification (J. R. Dorin, unpub-
lished) from the same cDNA library as CFA 8-9 using an
oligonucleotide probe for the published 5' end of the molecule
(Onink et al. 1987); (2) CFA 8-9 for the 11K calgranulin A
molecule (Dorin et al. 1987); and (3) pGAPDH10 (gift from
Dr Yvonne Edwards) for glyceraldehyde phosphate dehydro-
genase (Edwards et al. 1985), which is a housekeeping enzyme
expressed in all cell types, and therefore a suitable positive
control for the loading of intact RNA. Probes were labelled with
[α-32P]dCTP by random hexaodeoxynucleotide priming (Fein-

Results

Definition of antibody reactivities
The pattern of antibody binding to calgranulins A and B
was investigated by protein blot analysis of the two major
tissue sources: granulocytes and epithelial cells. Fig. 1
shows that CF145 recognizes the 11 K calgranulin A in
both granulocytes from chronic myeloid leukaemia (CML)
cells and in xenografted TR146 epithelial cells. CF557 reacts predominantly with the 14K calgranulin B from both tissues, though prolonged autoradiographic exposure reveals a slight cross-reactivity with calgranulin A. There is evidence from protein blot analysis under non-reducing conditions and from purification by gel
filtration that calgranulins A and B occur predominantly
as a heterodimer (data not shown).

Immunohistochemical analysis
The presence of the CFAg complex in polymorphonu-
clear leucocytes and in some monocytes from normal
peripheral blood, and its absence from lymphocytes, is
demonstrated by comparing indirect immunofluorescence
using monoclonal antibody CF557 together with
nuclear staining using the fluorescent DNA-intercalating
dye Hoechst 33258 (Fig. 2A,B). Cytoplasmic localiz-
ation, which is in keeping with the predicted nature of this
calcium-binding protein, is seen in more detail at a higher magnification (Fig. 2C), and has been confirmed

Expression pattern of CF-associated proteins
Table 1. Tissue distribution of calgranulins A and B

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal</th>
<th>Adult</th>
<th>Foetal*</th>
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<tr>
<td>Normal</td>
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<td>Brain</td>
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<td>Liver</td>
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<td>Pancreas</td>
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<td>Colon</td>
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<td>Jejunum</td>
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<td>Bladder</td>
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<td>Skin</td>
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<tr>
<td>Buccal smear</td>
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<td>Tongue</td>
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<td>Oesophagus</td>
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<td>Uterine cervixd</td>
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<td>Placenta</td>
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<td>Peripheral blood</td>
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<td>leucocytes:</td>
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<td>Granulocytesb,c</td>
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<td>Monocytesb</td>
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<tr>
<td>Lymphocytesb</td>
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<td>Abnormal</td>
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<td>Nasal polypb</td>
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<td>Inverted papilloma</td>
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<td>Skin</td>
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<tr>
<td>Psoriasis</td>
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<td>Squamous cell ca.</td>
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<td>Basal cell ca.</td>
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<td>Lung</td>
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<tr>
<td>Small cell ca.</td>
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<td>Squamous cell ca.</td>
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<td>Ditto xenograft</td>
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<td>Buccal</td>
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<td>Squamous cell ca.</td>
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<td>Ditto xenograft</td>
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* Both normal and CF foetal tissues tested; b normal and CF samples; c some hair follicles weakly positive but stratified epithelium and sweat glands negative; d may not have been completely normal sample; e polymorphonuclear cells; f cultured cells patchily positive; g cultured cell line also (more weakly) positive. ca, carcinoma.

been observed in CF (e.g. see Quinton, 1983). Low levels of patchy expression were seen in the epithelial cells of some of the hair follicles.

Because of the interest in skin, we also examined a series of abnormal skin sections in addition to normal samples and were surprised to note strong staining with both antibodies in a number of pathologic conditions associated with an accelerated turnover and proliferation of epithelial components. Among the calgranulin-positive conditions were psoriasis, eczematous dermatitis, some patches in graft versus host disease and squamous cell, but not basal cell carcinomas of the skin.

Lung is another tissue where abnormalities are invariably seen in CF as repeated bacterial pulmonary infections occurring from an early stage in the life of most CF patients (Talamo et al. 1983). Normal adult and foetal lung epithelia were completely negative with CF557 and CF145; the few positively staining tissue granulocytes served as useful internal positive controls in these sections. We had access to several foetal CF lung samples (from terminations of pregnancy following pre-
Fig. 3. Indirect alkaline phosphatase detection of calgranulins A and B in serial sections of adult tongue. A. With CF557; B, with CF145; C, with negative control antibody AFP. Arrowheads indicate the areas of staining in the upper layers of the squamous mucosa. Small arrows point to areas where tissue granulocytes show positive staining. Bar, 100 μm.

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Fig. 4. Indirect alkaline phosphatase detection of calgranulins A and B in the squamous cell carcinoma line TR146. Serial sections of xenograft tumours removed from the mouse host: A, with CF557; B, with AFP; C, immunofluorescence with CF145 on TR146 cultured cells, showing the reticular staining pattern reminiscent of cytoskeletal components. Bars, 40 μm.

carcinoma xenografts were negative. Subsequently, sections from the original tumour samples confirmed the xenograft data.

To allow us to manipulate epithelial cells that were likely to express the calgranulins, we acquired the human buccal squamous cell carcinoma line TR146. When cultured in vitro the cells were patchily and weakly positive initially, but strongly positive tumours were produced by xenografting into immuno-suppressed CBA mice (Fig. 4A,B). TR146 cells that had been in prolonged culture were shown by sensitive indirect immuno-
fluorescence to have a reticular network-like disposition of antigen (Fig. 4C).

One of the most frequently used sources of tissue for physiological demonstrations of the CF abnormality has been cultured airway epithelium from nasal polyps (e.g. see Welsh & Liedtke, 1986), since this tissue is available.
from both CF and unaffected individuals. Indirect alkaline phosphatase staining of sections from several non-CF patients (mainly patients suffering from atopic disease) and from one CF polyp showed no calgranulin reactivity in the epithelial cells; only infiltrating inflammatory cells were strongly positive. The only exception was one non-CF polyp sample, shown in Fig. 5A–C, which contained areas of strongly calgranulin-positive epithelium next to more normal-looking negative areas. On further inquiry, this turned out to be a neoplastic papillomatous lesion (inverted papilloma). Subsequently, the more sensitive indirect immunofluorescent staining showed very occasional weakly positive epithelial cells in the non-CF nasal polyp sections. This method of staining also revealed that a proportion (≈30%) of the cultured nasal polyp epithelial cells are weakly positive with both antibodies (Fig. 5D).

In none of the samples examined was connective tissue staining observed.

**Confirmation of calgranulin expression patterns by RNA probing**

Where RNA samples were available the cDNA probes for calgranulins A and B were used to assess expression of the two proteins compared with each other and with levels of expression of the glycolytic pathway enzyme glyceraldehyde phosphate dehydrogenase, which is used to monitor the quality and loading levels of the different RNA samples (Fig. 6). The pattern of expression at the RNA level mirrors faithfully the immunohistochemical findings. This confirms that antibodies and cDNA probes are monitoring the same components. Completely parallel expression in the tested tissues is observed for the two calgranulins.

In the myeloid lineage mature cells express both calgranulins as illustrated by two separate CML samples. HL60 cells induced to differentiate along the granulocyte pathway with dimethylsulphoxide or with retinoic acid also show hybridization with both probes, but uninduced HL60 does not (Dorin et al. 1987). One of the CFAg-positive myeloid somatic cell hybrids bearing chromosome 1 (lane 16), originally used for the chromosomal assignment of CFAg (van Heyningen et al. 1985; Dorin et al. 1987), expresses both calgranulins, while the negative control hybrid (lane 15), without chromosome 1, fails to express either. (This last observation is supporting evidence for the assignment of the 14K calgranulin B molecule to chromosome 1.)

*In vitro* cultured TR146 cells as well as xenografted TR146 tumour tissue were found to express both genes. RNA isolated from a freshly removed nasal polyp revealed a weak band with the probes only after very long autoradiographic exposure. This could be attributed to the presence of a minority of calgranulin-positive cells, either rare epithelial components (which have been seen on nasal polyp sections by immunofluorescence) or resulting from blood contamination of the surgically removed tissue. RNA from *in vitro* cultured epithelial cells of a human Wilms' tumour cell line (G401), a human osteosarcoma cell line (MNNG-HOS), or a human

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Burkitt's lymphoma cell line (DAUDI) failed to show hybridization with either probe. The levels of calgranulin A and B are similar except that in vitro cultured TR146 and retinoic acid induced HL60 cells express relatively more calgranulin B than A.

**Discussion**

When the cloning and sequence analysis of the CFAg components revealed that they belong to a family of calcium-binding proteins (Dorin et al. 1987), the obvious next step was to try to identify how they may be involved in the CF abnormality. The relationship between CF and the calgranulins is certainly not primary, since the disease locus has been clearly assigned to chromosome 7 (Kowulton et al. 1985; White et al. 1985; Wainwright et al. 1985), whilst the genes for calgranulin A (van Heyningen et al. 1985; Dorin et al. 1987) and calgranulin B (Bruggen et al. 1988; and as tentatively confirmed here) map to chromosome 1. We therefore had to consider: (1) the possible relationship between the calgranulins and the putative function of the CF gene; (2) whether the calgranulins are expressed in the cell and tissue types where the CF defect has been demonstrated; and (3) whether the CF gene might be functioning in cells, such as those of the myeloid lineage, where the calgranulins are clearly transcribed.

The CF gene on chromosome 7 is localized only by genetic and physical mapping using linked markers (Estivill et al. 1987). The gene itself has not been isolated and the most significant indications for function of the protein encoded at this locus come from physiological comparison of CF and normal cells, predominantly of epithelial origin from accessible tissues where clinical involvement has been demonstrated (Knowles et al. 1983). Whether the CF gene is expressed only in this restricted set of cell types will only be settled when the identity of its product is known. (There is plenty of evidence for highly tissue-specific effects of mutations in genes that are very widely expressed, e.g. the immunodeficiency caused by loss of function at the adenosine deaminase locus.)

The observation that chloride and sodium ion concentrations are abnormally elevated in CF sweat (see Talamo et al. 1983; the basis of the current diagnostic test for the disease) led to the demonstration of an impermeability to chloride ions in CF sweat glands (Quinton, 1983). The finding that beta-adrenergic responsiveness is defective in isolated whole CF sweat glands (Sato & Sato, 1984) was extended to the physiological demonstration of altered regulation of chloride channel opening in cultured airway epithelia from CF trachea and nasal polyps (tissues most closely approximating to lung, which is the site of the clinically most serious pathology in CF) (Frizzell et al. 1986; Welsh & Liedtke, 1986). The most recent data (Schoumacher et al. 1987; Li et al. 1988) suggest that the abnormality in cultured CF airway epithelial cells lies in the inability of these cells to respond to signals, such as beta-adrenergic stimulation, by the opening of the apical membrane chloride channels. The mechanism for cyclic AMP generation appears to remain unaltered; it is the ability to respond to phosphorylation signals from cyclic AMP-dependent protein kinase that has been abolished in CF cells. Individual steps along this (or most other) signal transduction pathway have not yet been identified.

If the calgranulins and the CF gene product do participate in the same signal transduction pathway, they are probably interacting components of the same multicomponent assembly, which are necessary for keeping signals specific (Neer & Clapham, 1988). Mutation in one component can lead to aberrant localization of associated molecules. The elevated serum levels of calgranulins in CF homozygotes and heterozygotes could be a manifestation of this. The absence of cytochrome b545 in chronic granulomatous disease is a good example of such a situation where the disease gene has been shown to encode an associated molecule, and it was the prior knowledge of the cytochrome abnormality that led to the understanding of the basic defect (Teahan et al. 1987).

The function of the family of calcium-binding proteins to which the calgranulins belong is largely unknown, but a role in signal transduction is an obvious possibility. A clear-cut function has been described only for 'p11', a protein that is the regulatory subunit of the heterotetrameric molecule formed by association with the 36K calpeptin I (Gerke & Weber, 1985; Glenney et al. 1986), which is identical to lipocortin II (Huang et al. 1986). This 36K protein is only one of a family of similar phospholipid-dependent calcium-binding proteins (Glenney, 1986). Calpeptin I/lipocortin II has been shown to be a phosphorylation target for the tyrosine kinase action of epidermal growth factor receptor (Pepinsky & Sinclair, 1986). This may be associated with the function of these glucocorticoid-regulated lipocortins as inhibitors of phospholipase A2 (Huang et al. 1986). The spectrum of calpeptin/lipocortin activities is consistent with a function as a major component of a signal transduction pathway, which in turn is a likely target for the basic CF mutation. Abnormalities in such a pathway, regulating the release of arachidonic acid, have been demonstrated in peripheral leucocytes and suggested as the basic defect in CF (Carlstedt-Duke et al. 1986).

As far as studies on calgranulin expression are concerned, our immunohistochemical analysis with indirect alkaline phosphatase staining did not reveal calgranulin in sweat glands of normal skin sections, or in normal or CF (adult or foetal) lung tissue, or in pancreas sections. Only in sections of simple mucous nasal polyp tissue did we see occasional epithelial cells expressing both calgranulins A and B, when using indirect immunofluorescence to reveal antigen. Cultured nasal polyp cells were found to be positive at higher frequency. However, the physiological signature of our cells was not monitored, so that the presence of the appropriate type of chloride channel in calgranulin-positive cells is not confirmed. Consistent calgranulin expression has now been observed in cultured nasal polyp and sweat gland epithelial cells (from both normal and CF donors) in which the presence of the correct type of chloride channel has been demonstrated (A. Hoogeveen, V. van Heyningen et al., unpublished data). We are now re-examining other CF 'affected' tis-
sues for calgranulin expression by the more sensitive immunofluorescence technique. The final judgement on the possible interaction between the calgranulins and the product of the CF gene is therefore not yet possible.

An alternative approach to the problem is to look for evidence of CF gene expression in tissues where calgranulins are clearly present. The most likely tissue source for the raised levels of calgranulins in CF homozygote and heterozygote serum (Hayward et al. 1987; Bruggen et al. 1988) is from granulocytes. This would imply that these cells of the myeloid lineage express the CF defect. aberrant beta-adrenergic responsiveness in CF homozygote and heterozygote granulocytes has been reported (Galant et al. 1981; Davis et al. 1983), but never followed up with the type of physiological analysis described above. Mature granulocytes are fragile cells with a very short half-life (6h) in vitro and in vivo. This makes physiological investigation extremely difficult. It also perhaps explains why calgranulin levels are elevated in a number of diseases with a strong inflammatory component (Hayward et al. 1987; Bruggen et al. 1988; Andersson et al. 1988). A 33K granulocyte-specific calcium-dependent phospholipid binding protein, with the characteristics of a member of the lipocortin family, has been described (Sato et al. 1985). A proportion of normal buccal cells also express the calgranulins, and altered calmodulin activity in TR146 cells is reminiscent of the submembranous, network-like subcellular distribution of the calgranulins in TR146 cells is reminiscent of the submembranous, cytoskeletally associated disposition of some members of the lipocortin family (Owens et al. 1984), in one case associated with the calgranulin homologue p11 (Osborn et al. 1988).

The tissue distribution we have observed for calgranulins A and B suggests that in addition to mature myeloid cells these calcium-binding proteins are expressed in a normally highly defined subset of proliferative epithelia: normal ones as in tongue, oesophagus, buccal cells and uterine cervix; and in pathologically abnormal ones as in psoriasis, eczema and finally in malignant squamous cell carcinomas of skin, lung and buccal origin. In some instances, as in the case of the cell line TR146, these could provide a valuable tissue source for the analysis of ion channel control if it could be demonstrated that the CF type of chloride channel is functional here.

For those with an interest in diagnostic pathology, immunohistochemical monitoring of calgranulin expression may also serve as a useful marker of skin disorders ranging from hyperproliferative states to malignancy.

We thank Dr Birgit Lane for the gift of the TR146 cell line and Dr Janet Wilson for the many nasal polyp samples. We are grateful to the MRC Unit’s photography department for their work on the illustrations. This work was supported in part by the Cystic Fibrosis Research Trust.

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Hayward, C., Glass, S., van Heyningen, V. & Brock, D. J. H.


(Received 18 May 1988 – Accepted 8 July 1988)