Expression of GATA family of transcription factors in T-cells, monocytes and bronchial biopsies

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ABSTRACT: GATA-binding proteins are a subfamily of zinc finger transcription factors with six members (GATA-1-6) that interact with the GATA deoxyribonucleic acid (DNA) sequence. This sequence is found in the regulatory regions of many genes including those encoding T-helper 2 (Th2)-like cytokines, receptors, adhesion molecules and enzymes, which may be important in the pathogenesis of bronchial asthma.

The expression of GATA-3, -4 and -6 was investigated in peripheral blood T-lymphocytes and monocytes and bronchial biopsies from 11 normal subjects and 10 steroid-naïve asthmatic patients.

Using Western blot analysis, T-cells from asthmatic subjects expressed 5 times the level of GATA-3 compared to that in normals. Confocal microscopy indicated that GATA-3 expression was both nuclear and cytoplasmic. GATA DNA binding complex containing GATA-3 was elevated in Th2 cells as determined by electrophorectic mobility shift assay. In contrast, monocytes from normal and asthmatic subjects expressed GATA-4 and -6 in equal amounts, but no GATA-3 was found. Using immunohistochemistry in bronchial biopsies, epithelial cells expressed high levels of GATA-3, GATA-4 and GATA-6 proteins. Comparison of Western blots of bronchial biopsies showed no significant differences between normal and asthmatic subjects.

In conclusion, the increased expression of GATA-3 in asthmatic T-cells may underlie augmented T-helper 2-like cytokines in this disease. However, the unaltered GATA-3 expression in epithelial cells suggests a distinct role for GATA-3 in these cells unrelated to T-helper 2-like cytokine release. Finally, no evidence was found for an increased expression of GATA-4 and GATA-6 in asthma.
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Asthma is characterized by chronic airway inflammation, with infiltration of T-lymphocytes, eosinophils, and monocytes/macrophages, and is associated with the increased expression of several inflammatory proteins, including cytokines, enzymes, receptors and adhesion molecules [1]. The molecular pathways involved in the induction of chronic cytokine expression and recruitment to the airways and activation of inflammatory cells in asthma are not well understood. However, there is increasing recognition that these processes involve increased transcription of inflammatory genes, and that this is regulated by transcription factors [2]. Several transcription factors are involved in asthmatic inflammation including nuclear factor-κB [3] and activator protein-1 [4].

The GATA family of transcription factors includes a family of zinc finger domain-containing proteins with six members (GATA-1-6) that share a common deoxyribonucleic acid (DNA)-binding motif (A/T)GATA(A/G). Differential gene regulation by the GATA family appears to be controlled, in part, by expression of specific GATA proteins in different cell types and in part by interaction (cross-talk) with other transcription factors. GATA-1 and GATA-2 proteins play a key role in the regulation of haematopoiesis [5]. GATA-3 is important in stimulating the expression of a number of T-helper 2 (Th2) cell-specific cytokines such as interleukin (IL)-4, IL-5 and IL-13 [6, 7] and in inhibiting the T-helper 1 (Th1) cytokine, interferon-γ [8]. GATA-4 has also been implicated in the control of IL-5 release in human T-cells [9]. More recently, GATA-4, -5, and -6 have been identified in nonhaematopoietic sites, including the gastrointestinal tract [10–13], which has a common embryological origin with the lungs. Furthermore, GATA-5 and -6 are expressed in various cell types in developing and adult mouse lungs [11, 13, 14]. GATA-6, independently or cooperatively with thyroid transcription factor-1, enhances murine surfactant protein A transcription [14, 15]. In addition, rhinovirus infection of A-549 cells upregulates the expression of vascular cell adhesion molecule 1 *via* increased activity of members of the GATA transcription factor family [16].

In order to determine the site of GATA-responsive gene expression, the authors have investigated the expression of GATA-3, -4, and -6 proteins in peripheral venous blood T-cells, monocytes and in bronchial biopsies of normal and asthmatic subjects.

Methods

Patients

Ten mild-to-moderate asthmatic patients who fulfilled the American Thoracic Society Criteria for asthma [17] and 11 age and sex-matched normal subjects were recruited (table 1). All asthmatic patients demonstrated a >15% improvement in forced expiratory volume in one second (FEV_1) following inhalation of 200 µg of salbutamol, and bronchial hyperresponsiveness (provocative concentration causing a 20% fall in FEV_1 PC_{20} methacholine <8 mg·mL^{-1}). All asthmatic patients were atopic as defined by two or more positive skin-prick tests to common allergens. All asthmatic patients had stable asthma and had not been receiving inhaled or oral corticosteroid therapy for ≥1 yr, and were using only inhaled β-adrenergic drugs intermittently for relief of breakthrough symptoms. Current smokers or exsmokers of >5 pack-yrs and patients with FEV_1 <75% pred were excluded. All normal subjects had normal lung function, negative skin-prick tests to common allergens (except for one subject), no bronchial hyperresponsiveness to methacholine (PC_{20} >32 mg·mL^{-1}) and were nonsmokers.

The present study was approved by the Royal Brompton Hospital Ethics Committee, and all subjects gave their informed consent.

Cell Culture

Primary epithelial cells were obtained by bronchial brushing exactly as described previously [18]. A549 cells and BEAS-2B cells were also cultured as previously described [19]. Human Th1 and Th2 cells were obtained from F. Sinigaglia (Roche-Milano, Milan, Italy) and were used subsequently for Western blotting, reverse transcriptase polymerase chain reaction (RT-PCR) or electrophoretic mobility shift assay (EMSA) analysis without stimulation. These cells have previously been shown to selectively produce Th1 and Th2 cytokines [20].

Peripheral venous blood monocytes and T-cell separation

Peripheral venous blood (100 mL) was collected (08:00–09:00 h) into sterile 60 mL syringes each containing 5 mL of 100% ACD (dextroglucose and disodium citrate solution). Monocytes were isolated by adherence to plastic as previously described [21] and were collected by scraping the wells. CD3+ T-cells were isolated from peripheral blood mononuclear cells (PBMCs) by negative selection of pan T-cells using a commercially available kit according to the manufacturer's instructions (Miltenyi Biotec, Bisley, UK). The pan isolation kit used for the experiments contain antibodies against CD16 and CD56 (expressed on human natural killer (NK) cells, but not T-cells) and this should also remove most of the CD3+ NK cells [22]. C-C Chemokine Receptor-5 positive (CCR5+) cells were further isolated from T-cells by immunomagnetic beads and subsequently analysed for GATA-3 expression by Western blotting.

Table 1. – Clinical characteristics of normal subjects and asthmatic patients

Subject No.	Age yrs	Sex	Skin-test	FEV_1 % pred	FVC % pred	PC_{20} mg·mL^{-1}
Normals						
1	28	M	neg	92	103	>32
2	33	M	neg	112	119	>32
3	37	F	neg	107	120	>32
4	32	M	neg	91	100	>32
5	27	M	neg	97	110	>32
6	27	M	neg	87	107	>32
7	24	F	neg	95	98	>32
8	30	F	pos	96	100	>32
9	22	F	neg	108	102	>32
10	23	F	neg	83	95	>32
11	24	F	neg	103	99	>32
Asthmatics						
1	38	F	pos	82	100	N/A
2	33	M	pos	86	98	N/A
3	43	M	pos	75	95	N/A
4	27	F	pos	77	96	0.36
5	24	F	pos	76	95	1.607
6	24	F	pos	98	102	0.21
7	42	M	pos	76	99	4.94
8	33	M	pos	96	102	1.14
9	26	F	pos	106	105	N/A
10	22	M	pos	98	107	4.6

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; PC_{20}: provocative concentration causing a 20% fall in FEV_1; N/A: not available; M: male; F: female; pos: positive; neg: negative.

Western blot analysis

Whole cell proteins were extracted from T-cells, monocytes and bronchial biopsies as previously described [3]. At least 20 µg·lane^{-1} of whole-cell proteins were subjected to a 10% or 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose filters (Hybond-Enhanced chemiluminescence (ECL), Amersham Pharmacia Biotech, Amersham, UK) by blotting. Filters were blocked for 1 h at room temperature in Tris-buffered saline (TBS), 0.05% Tween 20, and 5% non-fat dry milk. The filters were then incubated with mouse or goat antihuman GATA-3 (HG3-35, sc-269), GATA-4(C-20, sc-1237), -6(C-20, sc-7244) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature in TBS, 0.05% Tween 20, and 5% nonfat dry milk at a dilution of 1:500. These antibodies are specific for the respective human GATA proteins and do not cross-react with each other. Filters were washed three times in TBS, 0.05% Tween 20 (TBS-Tween) before incubating for 45 min at room temperature with antimouse or antigoat antibody conjugated to horseradish peroxidase (1:4,000; Dako, Ely, UK) in TBS-Tween and 5% nonfat dry milk. After a further three washes in TBS-Tween, visualization of the immunocomplexes was performed using ECL as recommended by the manufacturer (Amersham Pharmacia Biotech). As an internal control, each filter was reprobed with an antihuman actin antibody (Santa Cruz Biotechnology).

The bands, which were visualized at ∼43 kDa (actin), 49 kDa (GATA-3), 55 kDa (GATA-4) or 50 kDa (GATA-6) were quantified using a densitometer with Grab-It and GelWorks software (UVP, Cambridge, UK). The individual band optical density values for each lane of GATA-3, -4, and -6 were expressed as the ratio with the corresponding actin optical density value of the same lane.

Electrophoretic mobility shift assay and supershift assay

Proteins were extracted from cloned Th1 and Th2 cells using Reporter Lysis Buffer (Promega, Southampton, UK) according to the manufacturer′s instructions. Protein (10 µg) from each sample was preincubated at 4°C for 30 min in binding buffer (10 mM Tris HCl, pH 7.5, 1 mM $MgCl_2$, 0.5 mM ethylene diamine tetra-acetic acid (EDTA), 0.5 mM dithiothreitol (DTT), 50 mM NaCl, 4% glycerol, 0.1 µg·µL^{-1} salmon sperm DNA). Double-stranded oligonucleotides encoding the consensus GATA DNA binding sequence (5′-CACTTGATAACAG AAAG TGATAACTCT-) (Santa Cruz Biotechnology) were end labelled with [γ-^{32}P]-adenosine triphosphate (ATP) and T$_4$ polynucleotide kinase. Each sample was then incubated with 50,000 cpm of labelled oligonucleotide for 40 min at 4°C in the presence or absence of a 10-fold excess of unlabelled oligonucleotide. Protein-DNA complexes were separated on a 6% polyacrylamide gel using 0.25× Tris-Borate-EDTA running buffer. In some experiments, 50 µg protein from Th2 cell lines were incubated with 5 µg antiGATA-3 antibody (H-48, Santa Cruz Biotechnology), for 4 h at 4°C prior to addition of labelled double stranded oligonucleotide.

Fibreoptic bronchoscopy and processing of bronchial biopsies

Normal subjects and asthmatic patients attended the bronchoscopy suite at 08:30 h after having fasted from midnight and were pretreated with atropine (0.6 mg *i.v.*) and midazolam (5–10 mg *i.v.*). Fibreoptic bronchoscopy was performed as previously described [3]. Three or four bronchial mucosal biopsy specimens were taken from segmental and subsegmental airways of the right lower lobe. Bronchial biopsies for immunohistochemistry were immediately placed in ornithyl carbamyl transferase embedding media, then snap-frozen in isopentane, precooled with liquid nitrogen and stored at -70°C. Bronchial biopsies for Western blot analysis were immediately placed on ice and processed as described later. All biopsies were frozen within 20 min of collection. Five-micrometre sections were placed on poly-L-lysine coated microscope slides (Sigma, Poole, UK), air-dried for 30 min then wrapped in aluminium foil and stored at -70°C prior to immunostaining. Bronchial brushings were obtained and cells collected and stored as previously described [18].

Immunohistochemistry for GATA-3, -4, and -6 in the bronchial biopsies

Sections were fixed with cold 4% phosphate-buffered paraformaldehyde solution and washed repeatedly with phosphate-buffered saline (PBS). The sections were treated with 0.1% saponin in PBS. Endogenous peroxidase activity was blocked by incubating slides in 1% hydrogen peroxide (H_2O_2) and 0.02% sodium azide in PBS for 1 h, followed by washing in PBS. Nonspecific labelling was blocked by coating with blocking serum (0.1 M phosphate buffer containing 1% bovine serum albumin (BSA) and 10% normal swine serum) for 1 h at room temperature. After washing in PBS, the sections were incubated overnight at 4°C with a mouse monoclonal antihuman GATA-3 antibody (Santa Cruz Biotechnology). Alternatively, the sections were incubated with a goat polyclonal antihuman GATA-4 or GATA-6 antibody (Santa Cruz Biotechnology). All antibodies were used at dilutions of 1:100 and do not cross-react with other members of the GATA family.

For the negative control sections, normal mouse or goat immunoglobulins (Dako) were used at the same protein concentration as the primary antibody. After overnight incubation and repeated washing steps with PBS, the sections were subsequently incubated with antimouse or antigoat biotinylated antibody (1:200 dilution; Dako) for 45 min at room temperature. After further washing, the sections were incubated with avidin-horseradish peroxidase (1:200 dilution; Dako) for 45 min at room temperature. Slides were

then incubated with chromogen-fast diaminobenzidine for 5 min, after which they were counterstained in haematoxylin and mounted on mounting medium (Distrene 80, Dibutyl pthalate, Xylene (DPX); BDH, Poole, UK). As a positive control for GATA-3, sections from human lymph nodes were used.

Quantification

Counts of positive cells were made on all biopsy sections, and were divided according to whether the positive cells were in the airway epithelium or beneath the epithelium to a depth of 175 μm. Counts were made only in areas of intact epithelium. For GATA-3, -4, and -6 proteins, the number of positive cells was expressed as a percentage of nucleated cells in the epithelium and in the subepithelium in at least four fields at ×400 magnification. For the inflammatory cells, the number of positive cells was expressed as the number per field. For the epithelium, one field was defined as a length of 175 μm, and for the subepithelium one field was defined as an area of 175 μm^2. At least four fields were counted for each subject for the epithelium and subepithelium. An experienced observer made all counts, unaware of the clinical status or the origin of the sections.

Fluorescent immunocytochemistry for GATA-3, -4, and -6 proteins in T-cells and monocytes

T-cells ($1 \times 10^6 \cdot$ well^{-1}) were cultured in 24-well plates at 37°C, in 5% carbon dioxide (CO_2) and at 98% relative humidity (rH) for 18 h in 1 mL·well^{-1} of sterile Roswell Park Memorial Institute (RPMI) 1640 (Sigma) with added foetal calf serum (FCS) (10%), benzylpenicillin (0.1 mg·mL^{-1}), streptomycin sulphate (0.1 mg·mL^{-1}) and L-glutamine. After 18 h, the cells in each well were aspirated into 1.5 mL plastic tubes and microcentrifuged at 4°C, 12,000 rpm for 2 min. The cell pellet in each tube was resuspended with 20 μL of Hank's balanced salt solution (HBSS) and pipetted onto a sterile glass cover slip within the well of a six-well plate.

Monocytes ($5 \times 10^6 \cdot$ well^{-1}) were cultured on a sterile cover glass within each well of a six-well plate at 37°C, in 5% CO_2 and at 98% rH for 24 h in 1 mL·well^{-1} of sterile Dulbecco modified eagle's medium (Sigma) containing 10% FCS, benzylpenicillin (0.1 mg·mL^{-1}), streptomycin sulphate (0.1 mg·mL^{-1}) and L-glutamine.

The culture plates with T-cells or monocytes were left on ice to air-dry for 90 min and prefixed for 10 min with 2% formalin/PBS. The cell membranes were permeabilized for 10 min with 0.5% Nonidet P40/PBS. Nonspecific binding was blocked for 1 h at room temperature with 20% normal rabbit whole serum. After washing with PBS/0.1% BSA, the cells were incubated for 1 h at room temperature with mouse or goat antihuman GATA-3, -4, and -6 antibody (dilution 1:100; Santa Cruz Biotechnology) in PBS/0.1% BSA. As a negative control, the respective primary antibody was not added. After washing three times with PBS/0.1% BSA, the cells were incubated for 45 min at room temperature with rabbit antimouse (or antigoat) biotinylated secondary antibodies (dilution 1:100; Dako). After further washing with PBS/0.1% BSA, the cells were incubated for 45 min at room temperature with streptavidin conjugated with FITC (dilution 1:100; Dako). Finally, the cells were counterstained in haematoxylin and the cover glass mounted using citifluor and sealed with mounting medium (DPX; BDH). Slides were viewed using epifluorescence and confocal microscopy. Confocal scanning laser microscopy images were obtained with a Leica confocal microscope (Leica Microsystems, Milton Keynes, UK), equipped with a 488/514 nm dual band argon ion laser. An oil-immersion objective was used. Images were collected using a Leica confocal software analysis package (TCS-NT).

Ribonucleic acid extraction and reverse transcriptase-polymerase chain reaction for GATA-3

Total ribonucleic acid (RNA) was extracted using Qiagen ribonucleic (RNase) easy extraction kit following the manufacturer's instructions (Qiagen, Crawley, UK). RT-PCR was performed as previously described [23]. Primers for GATA-3 were TCCAA GACGTCCATCCACCAC and CGTGCCATCTCG CCGCCACAGT, giving a product of 243 base pairs (bp). PCR was performed in a Techne multiwell thermocycler (Techne, Cambridge, UK) at 94°C for an initial 1 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s. Final extension was for 5 min at 72°C (44 cycles for epithelial cells). The number of cycles was chosen after determination of the linear phase of the product amplification curve from serial sampling with increasing cycles of amplification. Products were distinguished by electrophoresis on a 2% agarose gel, ethidium-bromide stained and then visualized and photographed using ultraviolet luminescence.

Statistical analysis

Data are presented as mean±SEM. Differences between normal and asthmatic subjects were assessed with the Mann-Whitney U-test, and a value of p<0.05 was taken as statistically significant.

Results

T-lymphocytes

There were no significant differences in the numbers of T-cells ($27.9 \times 10^6 \pm 13.4 \times 10^6$ versus $26.3 \times 10^6 \pm 10.5 \times 10^6$ cells; n=10) or PBMCs ($190.5 \times 10^6 \pm 67.3 \times 10^6$ versus $164.9 \times 10^6 \pm 42.6 \times 10^6$ cells; n=10) isolated from 120 mL of peripheral venous blood from normal and mild-moderate steroid-naïve stable asthmatic patients.

Western blot analysis of isolated CD3+ T-cells from steroid-naïve asthmatic subjects showed a five-fold

higher expression of GATA-3, compared to normal subjects (0.86 ± 0.21 *versus* 0.17 ± 0.06; n=8, p<0.05) (fig. 1) suggesting the presence of increased numbers of Th2 cells. Confocal microscopy indicated that GATA-3 is found in the cytoplasm and nuclei of CD3+ T-cells isolated from both normal and mild/moderate steroid-naïve asthmatic subjects (fig. 1B and C). The number of cells expressing nuclear GATA-3 did not vary between the two subject groups. The authors were unable to detect GATA-4 and GATA-6 in T-cells either by Western blotting or by immunocytochemistry (data not shown). Using the chemokine receptor CCR5 as a marker for Th1 cells [24], the expression of GATA-3 in CCR5+ and CCR5- cells isolated from freshly isolated human T-cells was examined. GATA-3 was markedly elevated in CCR5- (Th2-like) cells compared with CCR5+ (Th1-like) cells (fig. 2a). Western blotting confirmed that Th2 cell lines contained more GATA-3 than Th1 cell lines (fig. 2b) and EMSAs confirmed that GATA-3 could bind DNA (fig. 2c). Using excess unlabelled GATA oligonucleotide, it was shown that the retarded band was specific, and supershift experiments with an antiGATA-3 antibody showed that this band contained GATA-3.

Monocytes

Using Western blot analysis in monocytes, the presence of the GATA-4 and GATA-6 proteins were observed, both in normal subjects and in asthmatic patients (data not shown). There was no difference in the expression of GATA-4 and GATA-6 proteins between the two groups of subjects. Human monocytes do not express GATA-3 proteins (data not shown). This was confirmed by immunocytochemistry, which indicated the presence of both GATA-4 and GATA-6 expression in normal and asthmatic subjects (data not shown). There was no difference in the expression or localization of GATA-4 or GATA-6 in either group. Confocal microscopy indicated that

GATA-4 protein staining was predominantly localized to the cytoplasm (data not shown).

Bronchial biopsies

Biopsies from asthmatic subjects showed increased staining intensity for major basic protein (MBP) and CD3, compared to normal subjects. Immunohistochemical staining (fig. 3) showed that most of the cells staining for the GATA-3 protein are bronchial epithelial cells, both in normal subjects and asthmatic patients. However, there are some GATA-3 positive cells in the *lamina propria* both in normal and asthmatic patients (fig. 3). Western blot analysis indicated that there is no significant difference in the expression of GATA-3 protein in bronchial biopsies of normal subjects compared with asthmatic patients (0.44 ± 0.092; n=6, *versus* 0.52 ± 0.09; n=8) (fig. 3). In contrast to a previous study which found no epithelial cell GATA-3 messenger ribonucleic acid (mRNA) [25], high levels of expression of GATA-3 protein within bronchial epithelial cells in biopsy specimens were detected. Therefore, GATA-3 mRNA expression in primary human bronchial epithelial cells was examined using RT-PCR. Primary cultures of human bronchial epithelial cells, along with cultured human lung epithelial cell lines (A549, BEAS-2BE), were found to express GATA-3 mRNA (fig. 3). Human Th2 cell lines were used as a positive control.

Immunohistochemical analysis also indicated that most of the cells staining for GATA-4 and GATA-6 proteins were bronchial epithelial cells, both in normal subjects and asthmatic patients (data not shown). However, there were some GATA-4 and GATA-6 positive cells, representing mainly monocytes/macrophages, in the *lamina propria* in both normal subjects and asthmatic patients. Using Western blot analysis, there was no significant difference between normal subjects and asthmatic patients in the expression of GATA-4 (2.5 ± 0.5; n=6, *versus* 2.1 ± 0.6; n=7) or GATA-6 (2.2 ± 0.8; n=6 *versus* 1.9 ± 0.3; n=7) in bronchial biopsies (fig. 3).
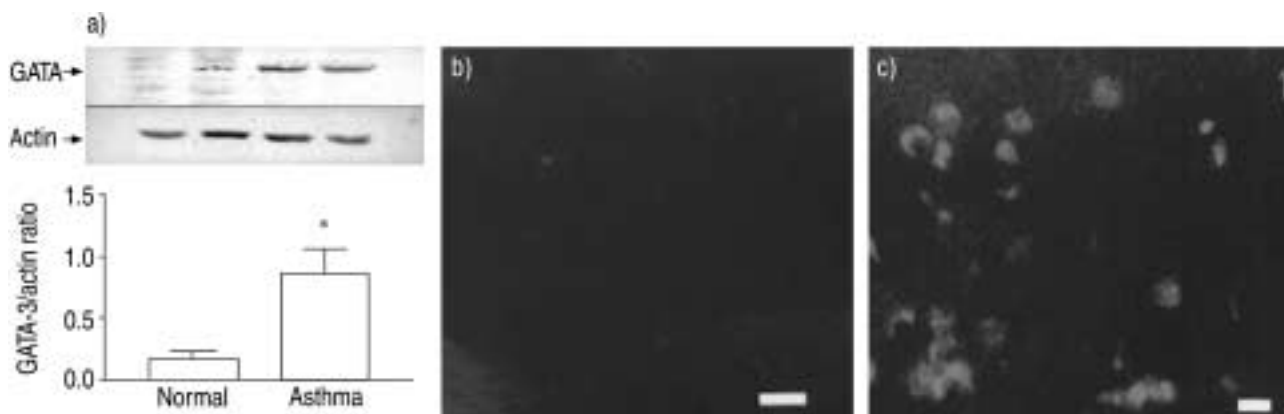

Fig. 1. – GATA-3 protein expression in T-cells of normal and asthmatic subjects. a) shows GATA-3 expression in T-cells isolated from asthmatic compared with normal subjects. The graphical representation of these results indicating increased GATA-3/actin ratio in T-cells from asthmatic subjects is shown below. Results are expressed as mean±SEM of eight subjects in each group. *: p<0.05. b) Confocal microscopy of GATA-3 localization in T-cells obtained from a normal subject indicates that GATA-3 is localized to both the cytoplasm and the nucleus. c) T-cells obtained from a steroid-naïve mild-asthmatic patient show GATA-3 staining epifluorescence in a group of GATA-3 positive cells. (Internal scale bar size=10 μm).
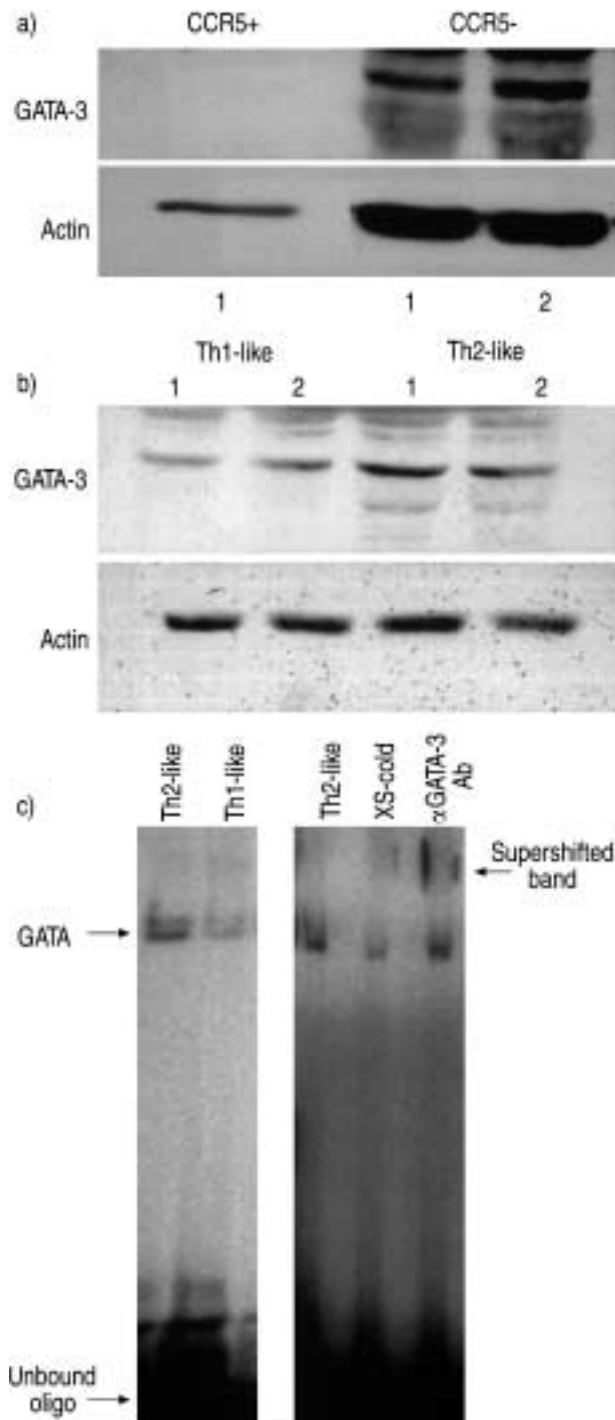
Fig. 2. – a) Western blot analysis of GATA-3 expression in freshly isolated human C-C Chemokine Receptor-5 positive (CCR5+) (T-helper 1 (Th1)) and C-C Chemokine Receptor-5 negative (CCR5-) (T-helper 2 (Th2)) T-cells. b) Western blot analysis showing increased expression of GATA-3 in human Th2 cell lines compared to Th1 cell lines. c) Electrophoretic mobility shift assay of total cellular proteins (10 µg) extracted from human Th1 and Th2 cell lines. Specificity was confirmed by the reduction in binding by preincubation with excess unlabelled probe (XS-cold). Incubation of Th2 protein with 5 µg antiGATA-3 antibody (αGATA-3 Ab) results in a supershifting of the retarded band. Unlabelled probe runs at the bottom of the gel. Figure is representative of three experiments.

Discussion

No significant differences were found in the number of PBMCs or T-cells in the peripheral venous blood of normal subjects and steroid-naïve stable asthmatic patients. These T-cells expressed GATA-3 protein, but not GATA-4 or GATA-6, and there was both increased expression in T-cells from asthmatic patients compared to normal subjects and increased DNA binding of GATA-3 in Th2 versus Th1 cells. This is in agreement with the results of a previous study which showed an increased expression of GATA-3 mRNA in the bronchial biopsies of patients with atopic asthma. In this study, the majority (∼60–90%) of GATA-3 mRNA expressing cells in asthmatic airways were CD3+ T-cells [25]. This probably reflects an increase in the Th2 cell population, however the possibility of GATA-3 expression in CD3+ NK cells, although unlikely, cannot be discounted.

In contrast to a previous study [25], the present study found that the predominant cell expressing GATA-3 was the bronchial epithelial cell in both normal subjects and asthmatic patients. The ability to detect both GATA-3 protein and mRNA in bronchial epithelial cells may have been due to differences in the techniques used (in situ versus PCR for mRNA). There is usually a good correlation between the presence of mRNA demonstrated by in situ hybridization and the localization of its protein. However, there are some instances where in situ hybridization failed to demonstrate the presence of specific mRNA for a given protein, but subsequently the presence of the protein was demonstrated using more sensitive techniques, such as RT-PCR. For example, using in situ hybridization HAMID et al. [26] were unable to demonstrate the presence of IL-5 mRNA. However a more recent study using RT-PCR and immunostaining showed that bronchial epithelial cells constitutively express both IL-5 mRNA and protein [27]. On the basis of the results presented here, the authors hypothesize that GATA-3 may play an important role in modulating the production of Th2-like cytokines in human T-cells in asthmatic subjects, but GATA-3 does not play an important role in the regulation of these genes in bronchial epithelial cells.

Interestingly, in normal subjects and asthmatic patients, T-cells did not express GATA-4 and GATA-6 proteins, whereas bronchial epithelial cells expressed GATA-3, -4 and -6 proteins. This suggests that differential gene regulation by the GATA family may be controlled in part by cell-specific expression of particular GATA proteins or by interaction with other cell-specific proteins. Thus, GATA-3 was found to play an important role in the differentiation of Th2 cells in conjunction with other transcription factors, such as nuclear factor of activated T-cells c/B [28], c-Maf [29] and STAT-6 [30].

Monocytes and bronchial epithelial cells from normal and asthmatic subjects expressed equal amounts of GATA-4 and GATA-6 proteins. These cells produce mediators such as granulocyte macrophage colony stimulating factor (GM-CSF), eotaxin-1 and IL-10, whose regulatory sequences contain
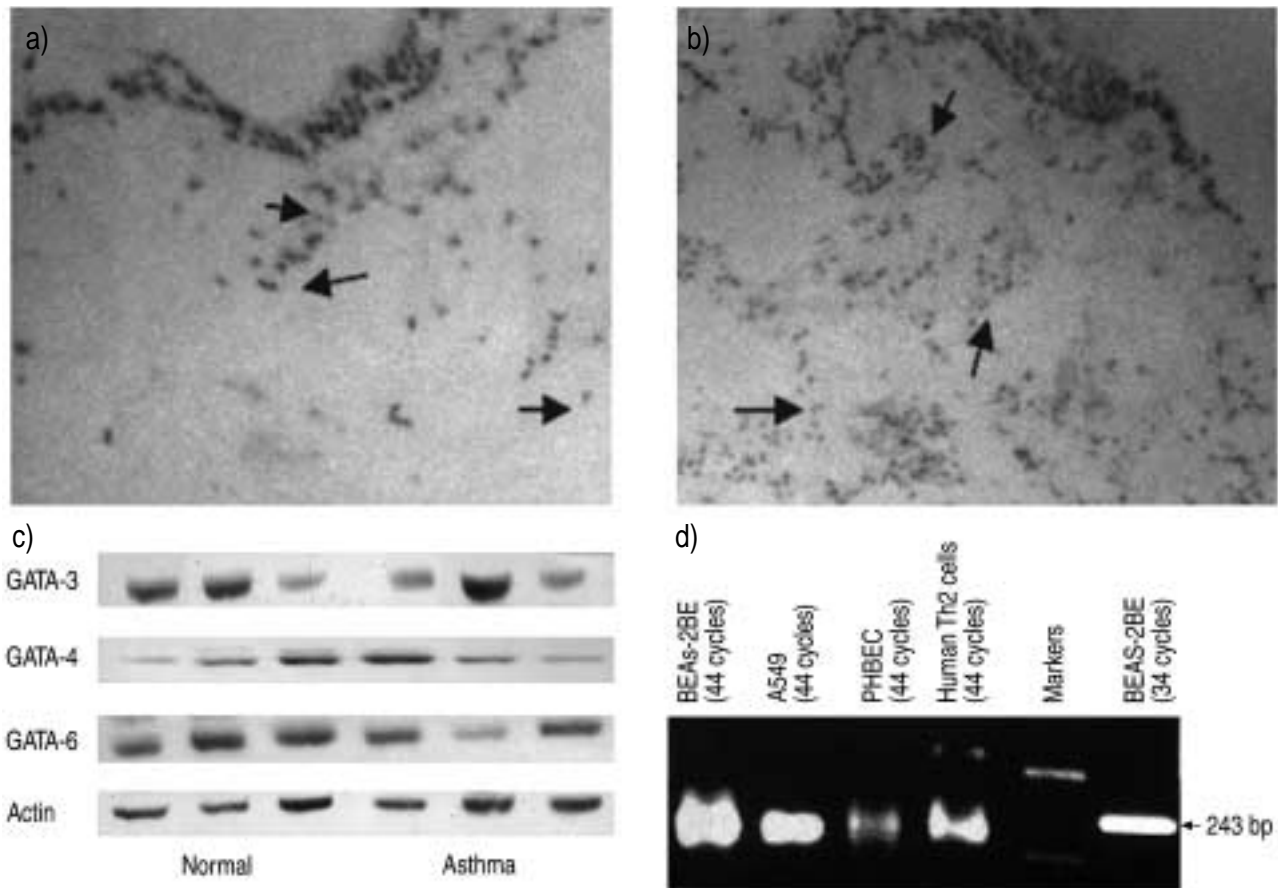
Fig. 3. – Immunohistochemistry and Western blot analysis of GATA protein expression in bronchial mucosal biopsies of normal and asthmatic subjects. The upper panels show immunohistochemical staining for GATA-3 protein in bronchial mucosal biopsies of normal (a) and asthmatic (b) subjects. Arrows indicate GATA-3 positive infiltrating cells. c) The expression of GATA-3, -4 and -6 protein and actin by Western blot analysis in bronchial biopsies of three normal and three asthmatic subjects. d) Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of GATA-3 messenger ribonucleic acid (mRNA) expression in primary human bronchial epithelial cells (PHBEC). The specific GATA-3 PCR product was detected in PHBEC after 44 cycles of PCR. As positive controls, GATA-3 mRNA expression was detected in human T-helper 2 (Th2) cell lines, BEAS-2BE cells and A549 cells after 34 cycles of PCR. The results are representative of three separate experiments. bp: base pairs.

GATA-binding sites. This suggests that although GATA-4 and GATA-6 proteins may be important in modulating inflammatory gene expression in these cells, they do not account for the differential expression of these mediators in asthma. Likewise, although GATA-6 may be important in the control of smooth muscle proliferation [31], acting *via* p21 cyclin-dependent kinase inhibitor protein (p21^{CIP}), this is not the case in human bronchial epithelial cells.

In summary, T-cells isolated from asthmatic patients expressed more GATA-3 protein compared to normal subjects, probably as a result of increased Th2 cell numbers. In contrast, monocytes did not express GATA-3, but expressed GATA-4 and GATA-6 proteins. There was no significant difference in expression between normal subjects and asthmatic patients. Bronchial epithelial cells expressed GATA-3, GATA-4 and GATA-6 proteins equally in normal subjects and asthmatic patients. The increased expression of GATA-3 in asthmatic T-cells, but not in bronchial epithelial cells, may underlie the augmented Th2-like cytokines observed in T-cells of asthmatic patients. No evidence was found for an increased expression of GATA-4 and GATA-6 in the airways of patients with asthma.

GATA-3 may play an important role in regulating T-helper 2-like cytokines in T-cells in asthma, but not in epithelial cells. Further studies are needed to characterize fully the role of the GATA proteins in the regulation of gene expression in T-cells, monocytes and bronchial epithelial cells and their potential role in the pathogenesis of asthma. At present, the ability of glucocorticoids to target GATA-3 action is unknown; and this may determine whether inhibition of GATA-3 activity may be an anti-inflammatory property of glucocorticoids [32].

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