METHODS

Study population

Fourteen patients (8 male and 6 female patients; age range, 20-54 years) were classified as having mild persistent asthma on the basis of clinical history and pulmonary function studies.[E1] All patients were atopic as documented by a positive skin test result for at least 1 aeroallergen. Inhaled corticosteroids were discontinued at least 4 weeks before bronchoscopy, and only short-acting β_2-agonists on demand were allowed. As a control group, we enrolled patients (14 male and 5 female patients; age range, 18-60 years) with a single lung lesion in whom the BALF used for the experiments was obtained from the contralateral side. They had no history of atopic diseases and at the time of bronchoscopy were free of respiratory symptoms and had forced vital capacity and FEV_1 values of greater than 90% of the predicted value.

Bronchoalveolar lavage procedure

A flexible fiberoptic bronchoscope (Olympus BF type P20; Olympus, Center Valley, Pa) was wedged into a segmental or subsegmental bronchus, and 3 fractions (50 mL each) of saline preheated at 37°C were introduced. Recovered fluid was pooled and filtered through 2 layers of sterile gauze. The fluid was then centrifuged twice at 800*g* for 10 minutes at 4°C and stored at −80°C for determination of PLA_2 activity and total protein content by a Bradford-based assay (Bio-Rad, Hercules, Calif).

Purification of human lung mast cells

Lung fragments chopped with scissors were dispersed into their cellular elements by means of enzymatic digestion with pronase (Calbiochem, San Diego, Calif), chymopapain (Sigma), collagenase, and elastase (Calbiochem). The mast cell suspension was enriched (>80%) by means of flotation over Percoll (Sigma) density gradients and then purified by using positive immunomagnetic selection with the CD117 Microbead Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Mast cell numbers and purity were determined by means of toluidine blue staining. The purity of mast cells used in the experiments was always greater than 98%.

RT-PCR

For semiquantitative PCR, equivalent templates of cDNAs were amplified by using target-specific primers for hGIB, hGIIA, hGIID, hGIIE, hGIIF, hGIII, hGV, hGX, hGXIIA, hGXIIB, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; see Table E1). The PCR products were separated on 2% agarose gel, stained with ethidium bromide, and visualized by using the image analysis system ChemidocXRS (Bio-Rad).

Real-time quantitative PCR (qPCR) was performed on the iCycler (Bio-Rad) with the Platinum SYBR Green qPCR kit (Invitrogen). Target-specific primers for hGIIA, hGIID, hGIIE, hGIIF, hGIII, hGV, hGX, and GAPDH suitable for qPCR were designed by using the Beacon Designer 3.0 (Bio-Rad; see Table E1). After an initial denaturation step at 94°C for 30 seconds, amplification was performed with 40 cycles of denaturation (94°C) for 30 seconds, annealing (55°C) for 30 seconds, and primer extension (72°C). The data were analyzed with iCycler iQ analysis software (Bio-Rad), and the mRNA signals in each sample were normalized to that of the GAPDH mRNA. The quantification of the mRNA target was obtained by calculating the relative expression of the reference gene compared with GAPDH. Data were expressed as Δ cycle threshold (Ct), which was calculated as the number of PCR cycles for liftoff for the target mRNA of interest minus the number of PCR cycles for liftoff for GAPDH mRNA: $\Delta Ct = Ct\,(\text{gene of interest}) - Ct\,(GAPDH)$. The Ct values for GAPDH were typically around 23. A ΔCt of less than 10 means high to medium expression, a ΔCt of 10 to 15 means medium to low expression, and a ΔCt of greater than 15 means low expression.

REFERENCE

E1. Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma—summary report 2007. J Allergy Clin Immunol 2007;120(suppl): S94-S138.

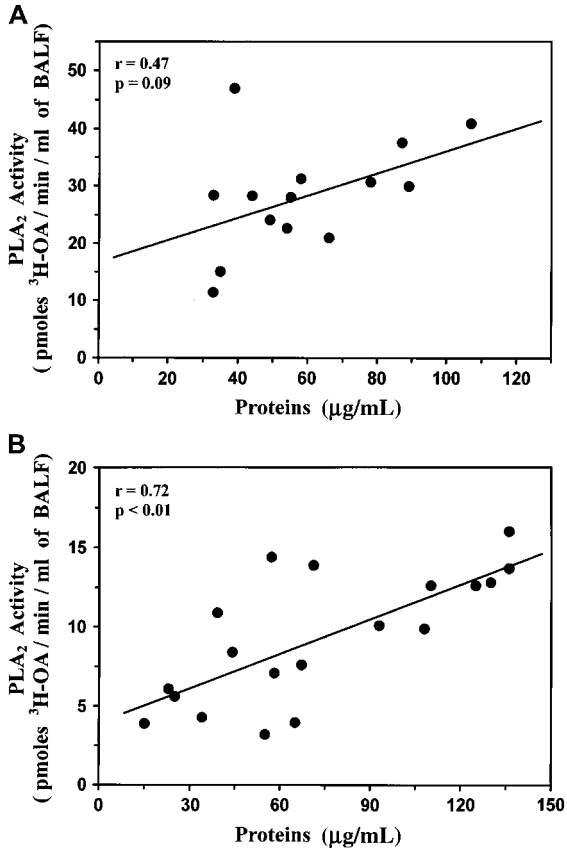

FIG E1. Correlation between PLA_2 activity and protein content in the BALF. Cell-free BALF from patients with bronchial asthma (**A**; n = 14) and control subjects (**B**; n = 19) was assayed for PLA_2 activity by using tritiated OA–labeled *E coli* membranes, as described in the Methods section, and for total protein content by using a Bradford-based assay. Data are plotted as a function of PLA_2 activity versus protein content. Correlation was assessed by using the linear regression function of Microsoft Excel software.

TABLE E1. Primer sequences and conditions for RT-PCR and qPCR

Target*	Product length (bp)	Ta	Primer (5′-3′)	GenBank accession no. or reference
hGIB (RT-PCR)	341	60°C	Forward: TCCTTGTGCTAGCTGTGCTG	Degousee et al[24]
			Reverse: TGAAGGCCTCACACTCTTTG	
hGIIA (RT-PCR)	434	57°C	Forward: ATGAAGACCCTCCTACTGTT	Degousee et al[24]
			Reverse: TCAGCAACGAGGGGTGCTCC	
hGIIA (qPCR)	117	55°C	Forward: ACCCTCCCTCCCTACCCTAAC	NM_000300
			Reverse: AATCTGCTGGATGTCTCATTCTGG	
hGIID (RT-PCR)	294	60°C	Forward: GAAAATGCCCATCCTCTCCT	Degousee et al[24]
			Reverse: AAACGCAGTCGCTTCTGGTA	
hGIID (qPCR)	85	55°C	Forward: CGGACTAGGTGGCAGAGG	NM_012400
			Reverse: GGTCTTCAGGTGGTCATAGC	
hGIIE (qPCR)	120	53°C	Forward: GGTGTTCCTTTGCCTCCTG	NM_014589
			Reverse: CCGCAGTAACAGCCATAGTC	
hGIIF (qPCR)	211	55°C	Forward: GACCCACACCCTCTCTCC	NM_0221819
			Reverse: GATGCTCTCCCTGCTTACG	
hGIII (RT-PCR)	500	60°C	Forward: TGCCTACAGAATCAGCACGA	Degousee et al[24]
			Reverse: TTGAGCAGCTGGAACTCGAT	
hGIII (qPCR)	100	55°C	Forward: GCGAGCAGCAGGGCAGAG	NM_015715
			Reverse: AGAATCTCCAACTCCACACCACAG	
hGV (RT-PCR)	358	60°C	Forward: TTGGTTCCTGGCTTGTAGTGTG	Degousee et al[24]
			Reverse: TGGGTTGTAGCTCCGTAGGTTT	
hGV (qPCR)	100	55°C	Forward: GCAACATTCGCACACAGTCC	NM_000929
			Reverse: CGGTCACAGGCACAGAGG	
hGX (RT-PCR)	370	60°C	Forward: GATCCTGGAACTGGCAGGAA	Degousee et al[24]
			Reverse: TCAGTCACACTTGGGCGAGT	
hGX (qPCR)	94	55°	Forward: GCTGCTGCTGCTTCTACC	NM_003561
			Reverse: CACAGTTCCTGCCAGTTCC	
hGXIIA (RT-PCR)	105	55°C	Forward: TGTTGTCAGGTGCCAGGAG	BC_017218
			Reverse: AAGGCGGCGTTCAGGTAC	
hGXIIB (RT-PCR)	115	55°C	Forward: GCCTGTGATTCCCTGGTTGAC	NM_032562
			Reverse: ACTCTTCCTTCTCCTCCTCTGC	
GAPDH (qPCR)	141	55°C	Forward: GTCCACTGGCGTCTTCAC	Granata et al[30]
			Reverse: CTTGAGGCTGTTGTCATACTTC	

*For PLA_2s, *h* stands for human, the Roman number after the letter G indicates the group, and the letter in caps after the number indicates the subgroup (eg, hGIB indicates human group IB PLA_2s).
Ta, Annealing temperature.