

Airway macrophages from patients with asthma do not proliferate

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Background: Macrophages are involved in asthma, but their pulmonary turnover is unknown. We compared the ability of bronchoalveolar lavage (BAL) and bronchial macrophages to proliferate in normal subjects and patients with asthma.

Methods: BAL cells from eight patients with asthma and eight normal volunteers were separated with a discontinuous Percoll gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). In a first experiment, nuclei of each alveolar macrophage (AM) fraction, stained with propidium iodide, were analyzed for DNA content with a flow cytometer, and the proportions of cells in the G₀/G₁, S, and G₂ + M phases were determined. In a second experiment, expression of Ki-67-related antigen was sought on AMs by immunocytochemistry. Macrophages from 10 patients with asthma and 10 normal volunteers were studied in biopsy specimens by means of immunohistochemistry with a panmacrophage monoclonal antibody (HAM-56) and a monoclonal antibody against proliferating cell nuclear antigen.

Results: The proportions of BAL AMs in the different phases of the cell cycle were similar in normal subjects and patients with asthma for all fractions, and the percentage of cells in S and G₂ ± M phases ranged from 7.3% to 11.3%. Under 1% of BAL AMs expressed Ki-67-related antigen. None of the macrophages present in the biopsy specimens expressed proliferating cell nuclear antigen.

Conclusions: This study does not indicate that an important source of airway macrophages is local proliferation. (*J ALLERGY CLIN IMMUNOL* 1993;92:869-77.)

Key words: Macrophages, asthma, cell cycle, DNA content, flow cytometry, immunohistochemistry

Airway macrophages are among the cells involved in the chronic inflammatory processes underlying bronchial asthma. Alveolar macrophages (AMs) obtained by bronchoalveolar lavage (BAL) from patients with asthma differ functionally and metabolically from those obtained from healthy subjects and have been shown to be in an activated¹⁻⁴ or postactivated stage. In bronchial biopsy specimens, the number of macrophages is signifi-

Abbreviations used

AMs: Alveolar macrophages
BAL: Bronchoalveolar lavage
PCNA: Proliferating cell nuclear antigen

cantly increased in patients with asthma, and these cells have been shown to be activated.^{5, 6} Recently, we extended the concept of macrophage heterogeneity to the AMs recovered by BAL in patients with asthma which showed an increased number of low-density cells,⁷ suggesting their previous in situ activation or a prolonged life span in the airways.

The origin of tissue macrophages is still not fully understood.⁸ Animal studies, in which steady-state conditions are used suggest that most macrophages are derived from monocytes that have migrated to tissues and differentiated. However, a smaller population of macrophages is apparently derived from locally dividing cells.⁹⁻¹⁴ During inflammation the number of DNA-syn-

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TABLE I. Characteristics of patients of BAL fluid study

Aas score	Sex	Age (yr)	FEV ₁ (% pred)	FEF ₂₅₋₇₅	Fluid recovery (%)	BAL fluid analysis				
						Cell number	AM (%)	Ly (%)	PMN (%)	EOS (%)
3	F	27	65	35	50	164	79	7	12	2
3	F	48	106	66	40	148	76	5	2	15
2	F	18	92	78	36	232	74	2	0	0
1	F	21	96	69	32	74	77	17	0	3
3	F	34	60	26	40	150	82	4	4	2
1	M	57	100	90	36	100	90	7	2	0
3	F	28	72	62	45	179	84	12	2	2
1	M	17	130	124	66	330	95	3	1	1

Cell numbers are given per microliter.

FEF₂₅₋₇₅, Forced expiratory flow, mid-expiratory phase; Ly, lymphocytes; PMN, polymorphonuclear neutrophils; EOS, eosinophils.

thesizing macrophages undergoes a transient rise,^{7, 15, 16} indicating a temporary rise in the local production of macrophages, although the share taken by local production is relatively small.¹⁷ In patients with sarcoidosis or pulmonary fibrosis and to a lesser extent in smokers, AMs display an enhanced mitotic activity, and an increased number of cells are found to be in the G₂ + M phase of the cell cycle.^{18, 19}

Among the possible methods of studying the cell cycle, flow cytometry is widely used to analyze the DNA content of a large number of cells on a cell-by-cell basis,^{20, 21} and such an analysis allows the determination of the cell proportion in the various phases (G₀/G₁, S, G₂ + M) of the cell cycle.²² It is also possible to study the cell proliferation by examining three proteins that are known to have an increase in expression in proliferating cells: the surface antigen transferrin receptor (Trf-r), the proliferating cell nuclear antigen (PCNA), and the nuclear antigen recognized by the monoclonal antibody Ki-67.^{23, 24} However, the relationships between these proteins and the cell cycle are not completely characterized yet, and the results that have been obtained are not completely comparable with those of other methods. Alternative methods may also be used, but they appeared to present some defects for the present study. The mitotic index only gives the percentage of cells in the M phase, and the tritiated thymidine labeling index only gives the percentage of cells in S phase.²⁵ Comparison of data showed that the cytometrically determined S phase proportions are generally higher than those obtained by the labeling index.²⁶ An approach similar to the labeling index is the bromodeox-

uridine incorporation revealed by a specific monoclonal antibody.²⁵

In an attempt to better characterize the origin of AMs in patients with asthma, AMs of eight normal subjects and eight subjects with asthma were studied after density fractionation; DNA content was analyzed by flow cytometry. In six patients with asthma and six control subjects the Ki-67-related antigen expression was also evaluated by immunocytochemistry. The second part of the study examined the proliferative capability of macrophages from bronchial biopsy specimens of 10 other patients with asthma and 10 other normal subjects with the use of PCNA monoclonal antibody. Macrophages were identified by use of a monoclonal antibody against a panmacrophage monoclonal antibody (HAM-56).

METHODS

Patients

Study 1. Eight patients with asthma (mean age $\pm$ SD: 31.3 $\pm$ 14.5 years) were selected according to the criteria of the American Thoracic Society (Table I).²⁷ All had a reversible airway obstruction as assessed by an increase of 20% of forced expiratory volume in 1 second (FEV₁) after inhalation of 200 μ g of salbutamol. The severity of asthma was quoted according to the score of Aas,²⁸ which is correlated with FEV₁, the activation of AMs, and the BAL eosinophil cationic protein level.²⁹ All patients underwent the same medical examinations including pulmonary function tests, allergy skin prick tests, RAST, and lung and sinus roentgenograms.

Eight normal volunteers (mean age $\pm$ SD: 40.7 $\pm$ 15.7 years) were enrolled in the study. None of them had any previous history of lung or allergic disease.

None of the subjects tested were smokers, and all

drugs that might have interfered with the performance of the study were carefully avoided. In particular, subjects were excluded if they had been treated with inhaled or systemic corticosteroids for a month; with sodium cromoglycate, nedocromil sodium, antihistamines, and/or theophylline for the past 2 weeks; or with β_2 -agonists for 48 hours before testing. All subjects had given their informed consent for the procedure, and this study fulfilled the criteria of the ethical committee of our hospital.

Study 2. Ten patients with asthma who ranged in age from 21 to 54 years (mean age $\pm$ SD: 41.3 ± 19.3 years) and 10 normal subjects who ranged in age from 19 to 47 years (mean age $\pm$ SD: 32.7 ± 12.6 years) were selected according to the criteria described above. All patients were at steady state.

Procedures: Study 1

Recovery of airway macrophages. Pulmonary cells were obtained by BAL as previously described.²⁹ The BAL fluid was immediately centrifuged (400 g, 4° C, 20 minutes), and the cell pellet was used for subsequent study. Viability was assessed by the exclusion of the trypan blue stain. Differential cell counts were performed on fixed cell preparations obtained by cytocentrifugation and stained by May-Grünwald Giemsa stain.

Density fractionation of airway macrophages. Unpurified BAL cells were layered onto a four-step Percoll density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) according to a modification of a method previously described.^{7, 30} The Percoll gradient solutions were prepared with piperazine-N,N'-bis[2-ethanesulfonic acid] buffer (Sigma Chemical Co., St. Louis, Mo.) for the dilution of Percoll to keep the pH constant. Percoll solutions with densities of 1.030, 1.040, 1.050, and 1.070 gm/ml were prepared. Each density solution was checked carefully by measuring the refractive index at 20° C with an ABBE refractometer (Zeiss, Jena, Germany). The refractive index was shown to be related to the density by means of a standard curve made of different dilutions of Percoll of known density determined by weighing each solution in a pycnometer at the same temperature on five distinct occasions. These different solutions were then layered into a 15 ml conical tube with a peristaltic pump (Minipulse 3, Gilson, France) at low speed, with the highest density solution at the bottom and the lowest at the top. The gradient was centrifuged at 400 g at 20° C for 30 minutes. The cells localized at each gradient interface were recovered, washed twice in RPMI 1640 (Flow Laboratories, Paisley, Scotland), and resuspended in a buffer consisting of RPMI with 10% dimethyl sulfoxide (Sigma) and 10% fetal calf serum (Flow Laboratories) at the concentration of 10^5 cells/ml. The percentage of macrophages was assessed by nonspecific esterase staining. The cells were kept frozen at -80° C until the DNA analysis was performed. In six patients with asthma and six normal subjects, a cytospin was also

performed on each AM fraction for Ki-67-related antigen analysis.

Flow cytometric DNA analysis. Each cell fraction was stained by propidium iodide according to the three-step procedure, including trypsin and spermin treatments, described by Vindelov et al.³¹ because it makes it possible to clean nuclei by splitting cytoplasmic proteins and to attain homogeneous staining of nuclei by modifying the chromosomal proteins. Briefly, the cells were incubated in the first step for 10 minutes with 1.8 ml of a solution of Nonidet P 40 (NP 40, Bethesda Research Laboratories, Bethesda, Md.), trypsin (Sigma), and spermin (Sigma) in phosphate-buffered saline. In the second step 1.5 ml of a solution of trypsin inhibitor (Sigma) and RNase (Sigma) in phosphate-buffered saline were added for 10 minutes. In the third step 1.5 ml of a solution of propidium iodide in phosphate-buffered saline was added for 15 minutes before analysis.

The samples were run in a flow cytometer (Cytofluorograf IIS; Ortho Diagnostic Instruments, Westwood, Mass.) equipped with a Coherent Innova 90-5 argon-ion laser (Coherent, Palo Alto, Calif.), which operated at a wavelength of 488 nm at 500 mW in a light-regulated mode. Before each run the cytometer was calibrated with fluorescent microspheres. Data from the forward angle scatter and fluorescence parameters (peak and area signals of fluorescence emission) were collected by a user-defined protocol and stored in an Ortho 2151 computer system (Ortho Diagnostic Instruments) in a list mode. The DNA-content histograms (red fluorescence emission area/number of cells per channel) were established from events gated on cytograms "red fluorescence area/peak" in order to eliminate doublets. The quality of the analysis was controlled by referring to cytograms "red fluorescence area/forward angle scatter." The cell proportions in the G_0/G_1 , and $G_2 + M$ phases were calculated from the DNA-content histograms according to the parametric method described by Baisch et al.³² All of the results were assessed by a graphic estimation of the cell proportions in each phase of the cell cycle.

Immunocytochemical staining with the monoclonal antibody Ki-67. The technique of immunocytochemical staining was derived from the work of Simony-Lafontaine et al.³³ Cytospin preparations of AM fractions were fixed in the 1:1 acetone-methanol mixture for 5 minutes at 4° C and rinsed with Tris-buffered saline. The slides were covered with a mouse monoclonal antibody against Ki-67-related antigen (Dako, Glostrup, Denmark) for 45 minutes at room temperature. The slides were washed and then incubated with a rabbit anti-mouse IgG (Dako) for 30 minutes at room temperature. The slides were then washed with Tris-buffered saline and incubated with alkaline phosphatase-antialkaline phosphatase complex (Dako). Then the chromogen substrate was added directly on each slide, incubated for 15 minutes, and counterstained with hematoxylin. A positive control for Ki-67-

TABLE II. Results of BAL

	BAL fluid study		Biopsy study	
	Asthmatics	Controls	Asthmatics	Controls
Cell number ($\times 1,000/\text{ml}$)	172 $\pm$ 8*	98 $\pm$ 58*	101 $\pm$ 47	94 $\pm$ 42
AMs (%)	82.1 $\pm$ 7.3	80.5 $\pm$ 12.5	86.0 $\pm$ 12.7	82.3 $\pm$ 9.8
Lymphocytes (%)	7.1 $\pm$ 5.1	7.1 $\pm$ 3.1	5.2 $\pm$ 6.7	6.8 $\pm$ 2.9
Neutrophils (%)	2.9 $\pm$ 3.9	4.5 $\pm$ 6.7	2.9 $\pm$ 1.8	3.9 $\pm$ 4.1
Eosinophils (%)	3.1 $\pm$ 4.9	0.6 $\pm$ 0.7	5.9 $\pm$ 5.9	0.5 $\pm$ 0.9

Statistical analysis by Mann-Whitney U test.

* $p < 0.003$.

related antigen staining was used and consisted of a non-small-cell carcinoma of the lung known to express Ki-67-related antigen in which 12% of cells were Ki-67-positive.³³ The positive cells were stained in red, and we used a negative control for each slide. We counted 200 cells on each slide and expressed the results as the percentage of positive cells.

Procedures: Study 2

Bronchial biopsies. Biopsies were performed with "alligator" forceps in subsegmental bronchi of the left lobe, and care was taken to avoid carinae because metaplasia may be observed on these sites according to a method previously described in detail.²⁹ The biopsy specimens were fixed in formaldehyde and embedded in paraffin blocks. Sections of two microns were cut and mounted onto slides.

Immunohistochemistry. The sections were deparaffinized and rehydrated in double-distilled water (vol/vol, -20°C). The slides were incubated with a highly specific mouse PC10 anti-human PCNA³⁴ (Dako) for 30 minutes at 37°C in humid chambers. Macrophages were studied with a panmacrophage monoclonal antibody (HAM-56, gift of Dr. Gown, Seattle, Wash.).^{6, 35, 36} Ham-56 is a sensitive, specific (with the exception of endothelial cells), and reliable marker of system macrophages in routinely processed biopsy specimens. The alkaline phosphatase-antialkaline phosphatase complex system³⁸ (Dako) was used to reveal immunohistochemical staining. Adjacent sections were stained with hematoxylin and eosin for routine pathologic examination.

Statistical analysis

Nonparametric tests were used to compare data expressed as means $\pm$ SD. The Mann-Whitney U test was used for nonpaired data, and the Wilcoxon W test was used for paired data. To determine the smallest increase in proliferation that should be detected, the type II error was calculated according to the method of Dixon and Massey³⁹ and with the use of the following formula: $1 - \beta = \delta/\sigma$, with δ being the maximal expected proliferation of macrophages in interstitial pulmonary fibrosis as reported by Bitterman et al.¹⁸ and σ

being the pooled estimate of the variance in our higher proliferative fraction. The power of the test is $1 - \beta$.

RESULTS

Study 1

The characteristics of the patients and the results of BAL are summarized in Tables I and II. Normal subjects had BAL fluid cells within normal range (Table II). The procedure was well tolerated by all the subjects without any exacerbation of their asthma.

The repartition of the cells from the Percoll gradients showed a significant increase in the light density fractions (first and second fractions) in the asthma group in comparison with the control group in which the cells were mainly found in the fourth fraction (Fig. 1). In both normal subjects and patients with asthma, the percentage of macrophages assessed by nonspecific esterase, which was found to be in the range of 72% to 82% in fraction 5, was always over 85% in fractions 2 to 4 and over 90% in fraction 1.

Proportions of AMs in the different cell cycle phases in different fractions are shown in Table III. Repartition of AMs in the cell cycle was not greatly different in patients with asthma and normal subjects. Cells in the G_0/G_1 phase ranged from 88.7% $\pm$ 9.5% to 92.1% $\pm$ 2.5%. Cells in the S and $G_2 + M$ phases ranged from 8.1% $\pm$ 3.95% to 11.3% $\pm$ 8.9%. In normal subjects cells in the $G_2 + M$ phase ranged from 3.6% $\pm$ 3.1% (fraction 4) to 1.34% $\pm$ 0.98% and 1.30% $\pm$ 1.0% (fractions 1 and 2). In patients with asthma cells in the $G_2 + M$ phase ranged from 3.02% $\pm$ 2.19% and 2.8% $\pm$ 1.1% (fractions 3 and 2) to 2.1% $\pm$ 0.9% (fraction 5). The power of the test was found to be 60%; thus, it is unlikely that this experiment would fail to detect a change of at least 15% in macrophage proliferation.

The expression of Ki-67-related antigen was observed only in fraction 3 of one patient with

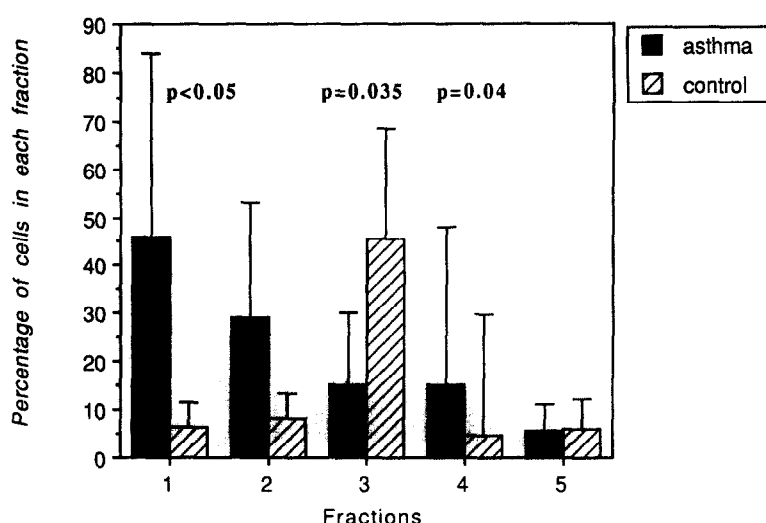


FIG. 1. Repartition of AMs in the density gradients (statistical analysis by Mann-Whitney U test).

TABLE III. Proportion of AMs in the different phases of the cell cycle

Phase	Fraction				
	1	2	3	4	5
G ₀ /G ₁					
Normal	89.2 ± 6.5	91.0 ± 4.4	88.7 ± 9.5	91.6 ± 4.4	90.5 ± 7.1
Asthma	90.8 ± 3.8	92.1 ± 2.6	89.9 ± 2.3	93.0 ± 1.8	90.0 ± 6.2
S					
Normal	9.5 ± 5.6	7.7 ± 3.6	9.6 ± 9.0	4.8 ± 3.7	8.6 ± 6.0
Asthma	6.7 ± 2.8	4.8 ± 2.8	7.1 ± 3.4	4.7 ± 2.2	8.2 ± 5.7
G ₂ + M					
Normal	1.3 ± 1.0	1.3 ± 1.0	1.8 ± 1.1	3.6 ± 3.1	1.4 ± 1.1
Asthma	2.4 ± 1.2	2.8 ± 1.1	3.0 ± 2.2	3.0 ± 2.2	2.1 ± 0.9
S + G ₂ + M					
Normal	10.9 ± 6.4	9.0 ± 4.4	11.3 ± 8.9	8.1 ± 2.9	10.0 ± 6.5
Asthma	9.0 ± 3.7	7.6 ± 2.5	10.1 ± 7.4	7.3 ± 7.4	10.3 ± 4.0

Results are expressed as means ± SD of percentages.

asthma and in none of the control subjects. One percent of AMs of this fraction was stained with the monoclonal antibody against Ki-67-related antigen. On the other hand, Ki-67-related antigen expression was found on the positive control slide.

Study 2

The characteristics of the patients are summarized in Table II and IV. The procedure was well tolerated by all the subjects without any exacerbation of their asthma.

Macrophages were observed in all biopsy specimens of normal subjects and patients with asthma as shown by the panmacrophage monoclonal antibody HAM-56. There were 23.4 ± 26.2 HAM-56-positive cells per square millimeter of

tissue in control subjects and a significantly greater number of cells (219.33 ± 282.2 cells/mm² tissue) ($p < 0.02$, Mann-Whitney U test) in patients with asthma. None of the control subjects demonstrated any PCNA staining. One patient with asthma demonstrated weak staining on epithelial cells.

DISCUSSION

This study confirms previous data on the heterogeneity of AMs in normal subjects and patients with asthma and indicates that the cell cycle distribution is similar in all AM fractions in patients with asthma and normal subjects, suggesting that AMs do not divide frequently. Ki-67-related antigen expression was almost entirely

TABLE IV. Characteristics of patients in biopsy study

Aas score	Sex	Age (yr)	FEV ₁ (% pred)	FEF ₂₅₋₇₅	Fluid recovery (%)	BAL fluid analysis				
						Cell number	AM (%)	Ly (%)	PMN (%)	EOS (%)
3	M	49	64	28	32	30	96	2	2	0
2	F	25	79	48	132	78	93	4	2	1
4	F	21	47	23	25	120	53	23	4	20
2	F	48	100	60	65	26	93	1	3	3
1	M	30	117	81	60	134	92	4	2	2
2	F	65	85	45	40	160	92	0	0	8
2	F	42	72	50	37	88	89	2	6	3
3	M	46	66	43	32	156	87	6	1	6
2	F	43	114	75	70	102	77	8	5	10
2	M	44	80	24	50	115	88	2	4	6

Cell numbers are given per microliter.

FEF₂₅₋₇₅, Forced expiratory flow, mid-expiratory phase; Ly, lymphocytes; PMN, polymorphonuclear neutrophils; EOS, eosinophils.

absent in patients with asthma and was completely absent in normal subjects. PCNA expression was absent in bronchial wall macrophages of normal subjects and patients with asthma.

It is difficult to analyze the mechanisms underlying the cell cycle in tissues because there is no marker that can assess the entire cycle, but it is easier to study the cell cycle distribution in fractionated cells.⁴⁰ For this reason, we have, in the first part of the study, examined AMs fractionated from BAL. The relationships between macrophages present in the airways and those in the BAL fluid are not completely characterized, but it appears that many cells recovered by BAL are bronchial cells.⁴¹ Moreover, many studies with AMs in asthma suggest their involvement in this disease. After *in vivo*^{42, 43} or *in vitro*^{44, 45} contact with specific allergen or nonspecific stimuli, AMs from patients with asthma have been shown to release lysosomal enzymes, prostaglandins (TxB₂),⁴⁶ leukotrienes,⁴⁷ and platelet-activating factor.⁴⁸ They are also able to generate oxygen free radicals after nonspecific stimulation.⁴⁹ Finally, Cluzel et al.⁴⁹ found a correlation among the severity of asthma, the release of oxygen free radicals by AMs, and the degree of eosinophilia in BAL fluids. In the present experiment we studied AMs fractionated by Percoll density gradient because: (1) over 97% of the cells of fractions 1 to 4 were shown to be AMs in a previous study⁷ and (2) there might have been some heterogeneity among monocytoid macrophages recovered in the higher density fractions, activated cells (fractions 2 to 4), and cells that have undergone a more advanced stage (fraction 1). However, there is no

clear relationship between the maturation and their density, and in most of the studies already published, cells of higher density were shown to be related to monocytes.

DNA content analysis by flow cytometry is a well established method.²⁰ The cells are stained with fluorescent dyes, which bind stoichiometrically and specifically to nucleic acids, so that the amount of fluorescence is directly proportional to the amount of DNA in each cell after RNA digestion by RNase. The cells fall into three categories: those that have an unreplicated complement of DNA and are therefore in the G₀ or G₁ phases, those that have a fully replicated complement of DNA and are in G₂ or M phases, and those that have an intermediate amount of DNA and are in the S phase. The Ki-67-related antigen expression was performed to further study proliferation because it is likely to identify the cells in S-G₂ phases, mainly in neoplastic cells.^{24, 33} However, it has been shown that the results obtained with the Ki-67 monoclonal antibodies in diploid cells do not correlate with those of other methods, whereas the correlation in aneuploid cells was significant.^{33, 50, 51}

Cells reproduce by duplicating their DNA content and then dividing in two. In the chromosome cycle, in which the nuclear DNA is duplicated, DNA synthesis alternates with mitosis. In eukaryotes, the cell cycle may be resolvable into several discrete stages. Quiescent cells are stable in the G₀ phase, but they can be stimulated into the G₁ phase by an appropriate stimulus. The DNA in the nucleus is replicated during only a limited portion of interphase, called the S phase, of the

cell cycle. This phase is followed by the G₂ phase, and arrest at G₂ phase before entering mitosis results in the appearance of polyploid cells, characteristic of cells that have undergone hypertrophy but cannot undergo division. Cells divide during a brief period known as the M phase. In asthma polyploid AMs are seen only very occasionally so that cells in the G₂M phase are likely to divide. Thus in the present study dividing cells constitute at most from 1.3% ± 1.0% to 3.6% ± 3.1% of cells from a given fraction. The results observed in normal subjects are close to those of a previously published study in which mitramycin dye staining was used.¹⁸ Although results of immunocytochemistry studies with Ki-67 monoclonal antibody do not completely correlate with results of flow cytometry, they confirm the lack of proliferation of AMs in both patients with asthma and normal subjects. With the use of mitramycin and flow cytometry¹⁸ or Ki-67-related antigen expression⁵² in active sarcoidosis^{18, 52} or pulmonary fibrosis,¹⁸ up to 7% of unfractionated AMs were shown to divide.

The selection of the antibody to characterize the cell cycle is difficult in tissue sections. The antibody against Ki-67 cannot be used in paraffin-embedded tissues and only recognized the S phase. The monoclonal antibody against PCNA reacts with a formalin-resistant epitope. The 48 and 34 kd nuclear proteins in PCNA are an accessory molecule for DNA polymerase-δ.³⁴ PCNA mostly recognizes cells in the S phase, but it can also be expressed in late G₁ and G₂-M phase cells.⁵³ Its expression in normal tissues correlates well with established parameters of cell proliferation such as the mitotic index,⁵⁴ Ki-67 immunoreactivity, bromodesoxyuridine immunostaining, and flow cytometric determination of S phase fraction. However, PCNA expression detected by monoclonal antibody may define different cell subpopulations in different cell types relative to those incorporating bromodesoxyuridine or expressing the target antigen for Ki-67.⁵⁵ It has been shown that cytokines may increase PCNA immunoreactivity,⁵⁶ but although their release is increased in asthma, they do not appear to play a role in the present model because PCNA-positive macrophages were not increased in asthmatic airways. Immunohistochemistry with PCNA monoclonal antibodies is one of the techniques from which to choose for use in paraffin-embedded tissues that are more suitable than frozen biopsy specimens for the study of endoscopic samples.

During a stable period of the disease, we only observed PCNA expression in 1 of 10 patients with asthma (vs 0 of 10 in control subjects), indicating that very few cells are proliferating. All sections contained macrophages as shown by immunostaining with the panmacrophage monoclonal antibody HAM-56.

Within any chronically inflamed tissue there is an increase in the numbers of macrophages, and this is particularly true in chronic lung inflammatory diseases.⁵⁷ Because of their central role in inflammation, attention has been focused on the mechanisms that result in an expansion of the macrophage population within inflamed tissue.^{8, 13-19} Tissue injury elicits an inflammatory response, one element of which is the activation of the local macrophage population¹⁷; in subacute inflammation in the rat there is an increased recruitment of monocytes.⁵⁸ In sarcoidosis it has been observed that the influx of macrophages was due both to replicating cells^{18, 52} and to an increase in monocyte migration from the peripheral blood.¹⁹ In bronchial biopsy specimens from patients with asthma, it has been observed that airway macrophages are increased in number and that many of these cells are bearing blood monocyte markers.^{5, 6} Metzger et al.⁴³ observed that after local allergen challenge, AMs are recently derived from blood monocytes because most of them were peroxidase-positive. Because the present study showed that AMs or macrophages present in the bronchial wall do not divide greatly in subjects with asthma, it may be postulated that in asthma most airways macrophages are derived from blood monocytes.

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