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Pulmonary Inflammation Monitored Noninvasively by MRI in Freely Breathing Rats

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A detailed analysis has been carried out of the correlation between the signals detected by MRI in the rat lung after allergen or endotoxin challenge and parameters of inflammation determined in the broncho-alveolar lavage (BAL) fluid. MRI signals after allergen correlated highly significantly with the BAL fluid eosinophil number, eosinophil peroxidase activity and protein concentration. Similar highly significant correlations were seen when the anti-inflammatory glucocorticosteroid, budesonide, manifested against allergen. In contrast, following endotoxin challenge, mucus was the sole BAL fluid parameter that correlated significantly with the long lasting signal detected by MRI. Since edema is an integral component of pulmonary inflammation, MRI provides a noninvasive means of monitoring the course of the inflammatory response and should prove invaluable in profiling anti-inflammatory drugs *in vivo*. Further, the prospect of noninvasively detecting a sustained mucus hypersecretory phenotype in the lung brings an important new perspective to models of chronic obstructive pulmonary diseases.

Key Words: **allergen; asthma; chronic obstructive pulmonary disease (COPD); edema; endotoxin; magnetic resonance imaging (MRI); lung; mucus; ovalbumin; lipopolysaccharide (LPS).**

Chronic diseases of the airways such as asthma and chronic obstructive pulmonary disease (COPD) involve a complex interplay of many inflammatory and structural cells, all of which release inflammatory mediators. Activated eosinophils are considered particularly important in asthma, contributing to epithelial cell damage, bronchial hyperresponsiveness, plasma exudation and oedema of the airway mucosa, as well as smooth muscle hypertrophy and mucus plugging, through the release of enzymes and proteins (1–5). A critical component of COPD is inflammation of the pulmonary mucosa and submucosa, characterised by an infiltration of the airways with neutrophils [for a recent review see (6)].

Animal models have been established in an attempt to mimic and study specific aspects of human respiratory disease (7). Actively sensitized Brown Norway (BN) rats exposed to allergen develop airway hyperresponsiveness and eosinophilic inflammation together with an increase in activated T cells (CD25+) in the airways (8–11), and thus reflect the key features of asthmatic inflammation. Similarly, an inflammation similar to that observed in COPD patients can be elicited in rodents by the administration of endotoxin (lipopolysaccharide; LPS), a bacterial macromolecular cell surface antigen. LPS activates mononuclear phagocytes through a receptor-mediated process, leading to the release of a number of cytokines, including tumor necrosis factor-α (TNF-α) (12, 13). TNF-α increases the adherence of neutrophils to endothelial cells, thus facilitating a massive infiltration of neutrophils into the lung (14). The inflammatory status of the lungs in these models is usually determined by broncho-alveolar lavage (BAL) fluid analysis.

We have recently described the use of magnetic resonance imaging (MRI) to investigate non-invasively the development of an edematous signal in the lungs of actively sensitized BN rats following challenge with allergen (15), and to follow the course of endotoxin-induced mucus hypersecretion in rat lung (16). We here present a detailed analysis of the correlation between the signals detected by MRI in the rat lung following allergen or LPS exposure and the individual parameters of inflammation determined in the BAL fluid. We have also included data from allergen-challenged rats treated with the glucocorticosteroid, budesonide.

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MATERIALS AND METHODS

Animals

Male BN rats (Iffa-Credo, L'Arbresle, France) weighing ca. 250 g were used. They were housed in a temperature- and humidity-controlled environment, having free access to standard rat chow and tap water. All experiments were carried out according to the Swiss federal regulations for animal protection.

Ovalbumin Sensitization and Challenge

Sensitization protocol. Ovalbumin (OA, 20 μg ml^{-1}) was mixed with aluminium hydroxide (20 mg ml^{-1}) and injected (0.5 ml per animal s.c.) together with *Acullulare pertussis* adsorbat vaccine (0.2 ml per animal i.p., diluted 1:4 with saline 0.9%) on days 1, 15 and 22.

Antigen exposure. On day 29 following the first injection of allergen animals were anaesthetised with 4% Forene (Abbott, Cham, Switzerland). OA (0.3 mg kg^{-1} dissolved in 0.2 ml saline) or vehicle (saline, 0.2 ml per animal) was administered i.t. and the animals allowed to recover.

Budesonide treatment. Some rats were treated with the glucocorticosteroid budesonide (1 mg kg^{-1}, i.t.) or vehicle (0.2 ml i.t.) 1 h before and 24 h post challenge with OA.

LPS Challenge

Nonsensitized animals were anaesthetised with 4% Forene, and LPS from *Salmonella typhosa* (Sigma, Dorset, UK; 1 mg kg^{-1} dissolved in 0.2 ml saline) or vehicle (saline, 0.2 ml) administered i.t.

Bronchoalveolar lavage (BAL). A detailed description of the BAL procedure and the analysis of the parameters of inflammation is provided in (15, 16). Briefly, animals were killed with an overdose of pentobarbital (250 mg kg^{-1} i.p.) immediately after an MRI examination. The lungs were lavaged, and the following parameters assessed in the BAL fluid: eosinophil, neutrophil, macrophage and lymphocyte numbers, eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities and protein concentration. In LPS-challenged animals, TNFα and mucus concentrations were also determined.

MRI. Measurements were carried out with a Biospec 47/40 spectrometer (Bruker, Karlsruhe, Germany) operating at 4.7 T, equipped with an actively shielded gradient system. The operational software of the scanner was Paravision (Bruker, Karlsruhe, Germany). A gradient-echo sequence with repetition time (TR) 5.6 ms, echo time (TE) 2.7 ms, band width 100 kHz, flip angle of the excitation pulse approximately 15°, field of view (FOV) 6 $\times$ 6 cm^2, matrix size 256 $\times$ 128 and slice thickness 1.5 mm was used throughout the study. A single slice image was obtained by computing the 2DFT of the averaged signal from 40 individual image acquisitions and interpolating the data set to 256 $\times$ 256 pixels. There was an interval of 530 ms between individual image acquisitions, resulting in a total acquisition time of 50 s for a single slice. The entire lung was covered by 28 consecutive slices. A birdcage resonator of 7 cm diameter was used for excitation and detection. During MRI measurements, rats were anaesthetised with 2% Forene in a mixture of O_2/N_2O (1:2), administered via a face mask. The body temperature of the animals was maintained at 37°C. Total examination time per animal, including positioning, was 25 min.

Data Analysis

The volume of signals appearing in the lung following OA or LPS challenge was quantified using a semiautomatic segmentation procedure implemented in the IDL (Interactive Data Language Research Systems, Boulder, CO) environment (version 5.1) on an SGI O2 (Silicon Graphics Inc., Mountain View, CA) system. Images were first weakly lowpass filtered with a Gaussian profile filter and then transformed into a set of four grey level classes using adaptive Lloyd-Max histogram quantization (17). This method avoided operator bias due to arbitrary choice of threshold levels on each image. Since the edema was comprised of high signal intensities in the original images, it was represented by the highest grey level class in the transformed images. This class could be extracted interactively by use of a region grower. Because of the unknown extent of the edema, no morphology parameters were incorporated in the region growing process. Instead, a contour serving as a growing border was drawn to control region growing manually.

The segmentation parameters were the same for all the analyzed images, chosen to segment regions corresponding to high intensity signals. Since the lung signals due to allergen or endotoxin challenge were of comparable intensity to those of vessels, the volume corresponding to the vessels was assessed on baseline images and then subtracted from the volumes determined on post-challenge images.

Statistical Analysis

The relationships between MRI signal volumes and inflammatory parameters were analysed using simple liner regression analysis (Origin version 6.1). A P value <0.05 was considered statistically significant.

RESULTS

OA Challenge

Figure 1a shows transverse sections through the chest of the same rat, acquired before and 24 h after challenge with OA (0.3 mg kg^{-1} i.t.). The intense and diffuse signal appearing in the lung following OA challenge is related to edema formation as has been described elsewhere; no edema was observed after application of saline (15). The maximum volume of the signal, 1.67 $\pm$ 0.23 ml (mean $\pm$ sem, n = 6), was observed 48 h after OA. The signal was present for approximately 100 h. The correlations between the signal volumes detected by MRI in the lung and the individual inflammatory parameters in the BAL fluid recovered from the same animals immediately after MRI acquisition, are summarized in Fig. 1b and Table 1. The MRI signals were highly significantly correlated with all inflammatory parameters. However, the strongest correlations were observed between the MRI signals and eosinophil numbers, EPO activity and the protein concentration (Table 1).

Treatment with budesonide (1 mg kg^{-1} i.t.) 1 h before and 24 h after OA challenge (0.3 mg kg^{-1} i.t.) reduced the volume of edematous signals detected by MRI at 48 h post-challenge to 0.23 $\pm$ 0.15 ml (mean $\pm$ sem, n = 6). Correlations between the volume of MRI signals and the inflammatory parameters assessed in the BAL, 48 h after OA instillation, are summarized in Table 2.

LPS Challenge

Figure 2 shows an image from a non-sensitised rat, 48 h after an i.t. administration of LPS (1 mg kg^{-1}). As has been described in (16) the signals appearing in the lungs as a consequence of the LPS challenge are dis-

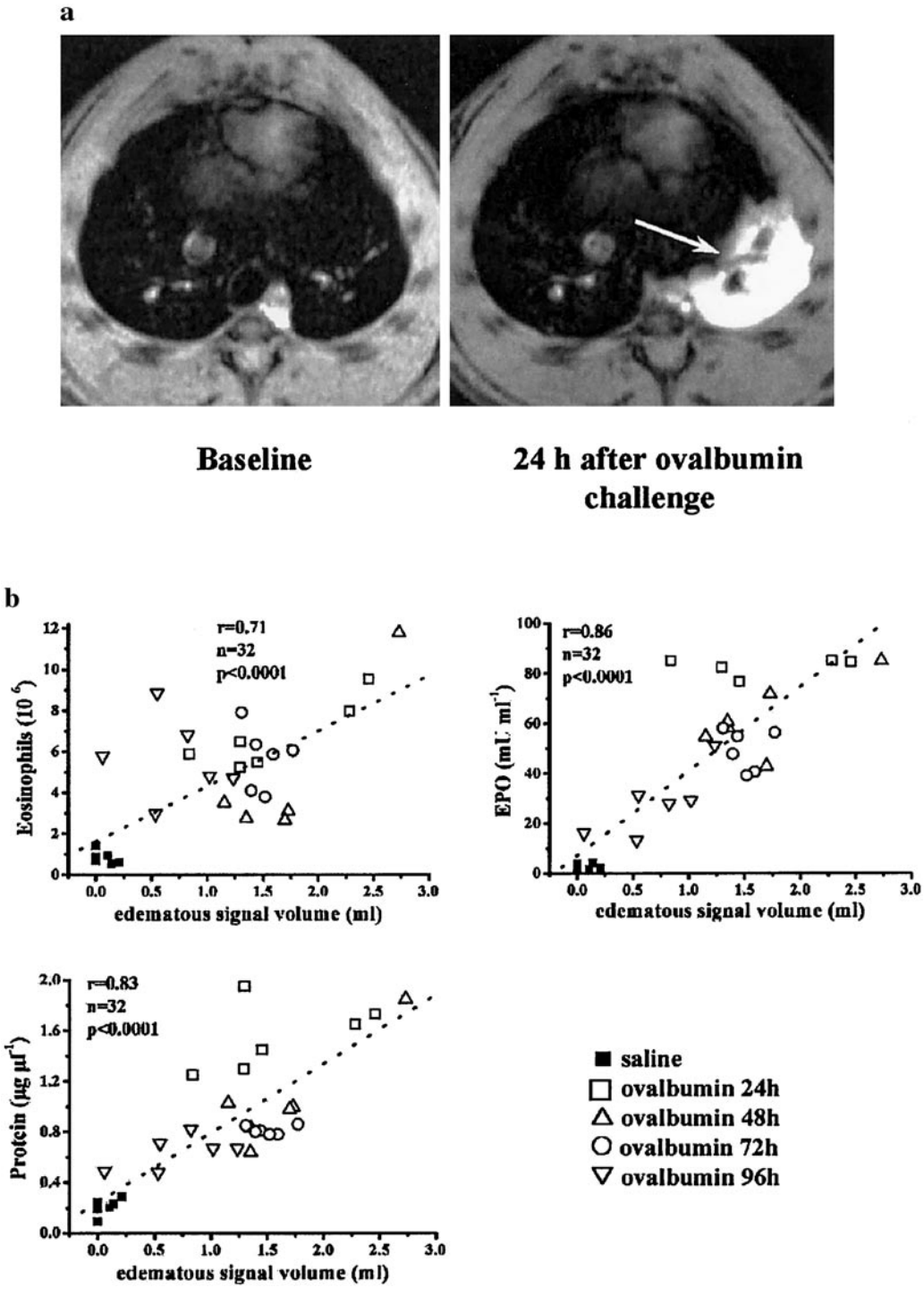

FIG. 1. (a) Transverse sections through the chest of a rat obtained before (left) and 24 h after (right) an intratracheal instillation of OA (0.3 mg kg^{-1}). Neither cardiac nor respiration triggering was applied, and the animal could respire freely during image acquisition. The arrow indicates the edematous signal. (b) Correlations between signal volumes detected by MRI in the lungs and the eosinophil numbers, EPO activity and protein concentrations assessed in the BAL fluid, at several time points from 24 to 96 h after administration of allergen or vehicle.

continuous and significantly less intense than those detected after OA challenge (Fig. 1). In general, either weak (MPO activity, macrophage number) or no (EPO activity, protein, eosinophil and neutrophil number, TNF-α) correlations were detected (Table 3). The notable exception, however, was the mucus concentration in the BAL fluid which correlated highly significantly with the MRI signals.

TABLE 1

Correlations between the Edematous Signal Volumes Detected by MRI in the Lungs of Sensitised BN Rats 24 to 96 h after Challenge with OA (0.3 mg/kg i.t.) and Inflammatory Parameters Assessed in BAL Fluid Recovered Immediately after the MRI Measurement

Inflammatory parameters	Range	*r*	*P*
EPO (mU ml^{-1})	0.92–85.02	0.86	<0.0001
Protein ($\mu g\ \mu l^{-1}$)	0.09–1.95	0.83	<0.0001
Eosinophils (10^6 cells/12 ml)	0.53–11.79	0.71	<0.0001
MPO (mU ml^{-1})	18.5–844.1	0.62	0.00014
Lymphocytes (10^6 cells/12 ml)	0.13–8.13	0.58	0.0005
Neutrophils (10^6 cells/12 ml)	0.1–18.13	0.53	0.0016
Macrophages (10^6 cells/12 ml)	3.92–22.3	0.37	0.038

Note. Edematous signal volumes ranged between 0 and 2.73 ml. The number of correlated samples was 32.

DISCUSSION

The methods currently used for evaluating the efficacy of potential treatments for respiratory diseases such as asthma or COPD in small animal models are generally invasive. BAL fluid analysis, in which the levels of inflammatory cells and their activation are determined, is one of the most commonly used approaches to define the inflammatory status of the lung. Recently we have described an MRI technique to monitor non-invasively the level of pulmonary inflammation (15, 16). In the present work, we carried out a detailed analysis of the correlation between the signal volume detected by MRI in the lungs of rats challenged either by allergen or LPS and the results of BAL fluid analysis in order to determine which inflammatory parameters are more closely reflected in the macroscopic MRI signals.

The model of exposing actively sensitized BN rats to ovalbumin shows many of the features of clinical asthma and has been used extensively to investigate the underlying pathophysiology of the condition (8, 18). Our results show that all inflammatory parameters determined in the BAL fluid were significantly corre-

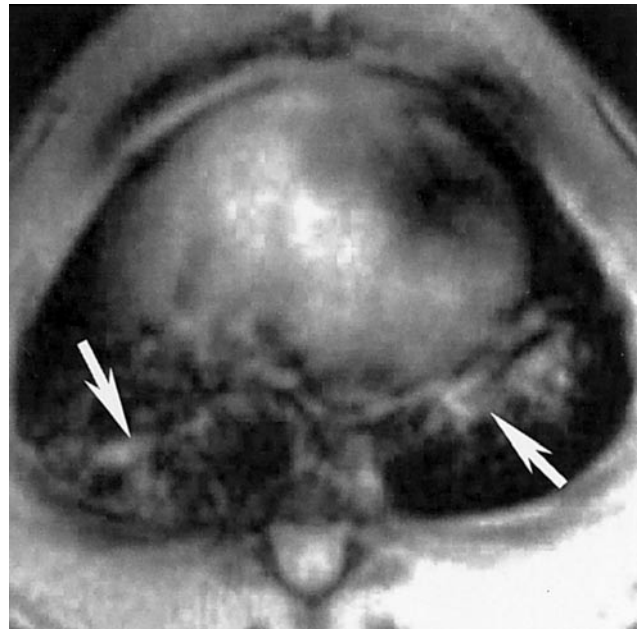

FIG. 2. Transverse section through the chest of a rat acquired 48 h after an intratracheal instillation of LPS (1 mg kg^{-1}). Neither cardiac nor respiration triggering was applied, and the animal could respire freely during image acquisition. The arrows indicate signals related to secreted mucus, as demonstrated in (16).

TABLE 2

Correlations between the Edematous Signal Volumes Detected by MRI in the Lungs of Sensitised BN Rats 48 h after Challenge with OA (0.3 mg kg^{-1} i.t.) and Inflammatory Parameters Assessed in BAL Fluid

Inflammatory parameters	Range		*r*	*P*
	Vehicle	Budesonide		
EPO (mU ml^{-1})	0.92–84.94	3.9–48.4	0.93	<0.0001
Protein ($\mu g\ \mu l^{-1}$)	0.09–1.85	0.22–0.5	0.96	<0.0001
Eosinophils (10^6 cells/12 ml)	0.53–11.79	1.28–3.67	0.79	<0.0001
MPO (mU ml^{-1})	18.5–296.4	11.9–186.4	0.94	<0.0001
Lymphocytes (10^6 cells/12 ml)	0.13–3.85	0.41–1.64	0.82	<0.0001
Neutrophils (10^6 cells/12 ml)	0.1–2.66	0.18–1.26	0.81	<0.0001
Macrophages (10^6 cells/12 ml)	3.92–7.77	2.42–6.88	0.46	0.046

Note. Animals were treated with either vehicle (0.2 ml i.t.) or budesonide (1 mg kg^{-1} i.t.), 1 h prior to and 24 h after the challenge. Edematous signal volumes ranged between 0 and 2.73 ml, and 0 and 0.77 ml for saline- or budesonide-treated rats, respectively. The number of correlated samples was 19.

TABLE 3

Correlations between the MRI Signal Volume Detected in the Lungs of Naïve BN Rats at Various Time Points (24 to 384 h) after Challenge with LPS (1 mg kg^{-1} i.t.) and Inflammatory Parameters Assessed in the BAL Fluid

Inflammatory parameters	Range	*r*	*P*
EPO (mU ml^{-1})	0.81–17.57	0.31	0.065
Protein ($\mu g\ \mu l^{-1}$)	0.16–0.53	0.27	0.12
Eosinophils (10^6 cells/12 ml)	0.3–6.05	0.29	0.08
MPO (mU ml^{-1})	5.6–418.47	0.33	0.049
Neutrophils (10^6 cells/12 ml)	0.3–124.83	0.23	0.19
Macrophages (10^6 cells/12 ml)	2.68–20.42	0.34	0.04
TNF-α ($\mu g\ ml^{-1}$)	0–276.67	0.03	0.88
Mucus ($\mu g\ ml^{-1}$)	0.15–53.73	0.76	<0.0001

Note. The signal volumes ranged from 0.022 to 0.957 ml. The number of correlated samples was 36.

lated to the MRI signals, assessed 24 to 96 h after OA instillation. However, three of them, namely eosinophil number, EPO activity (a marker of eosinophil activation) and protein concentration (a marker for plasma extravasation into the lungs), were particularly highly correlated with the MRI signals. Eosinophil infiltration is a characteristic feature of asthmatic airways and differentiates asthma from other inflammatory conditions of the airways. Allergen administration to sensitized BN rats results in a marked increase in eosinophils in BAL fluid (18), and a significant correlation exists between bronchial responsiveness and eosinophil counts in the BAL fluid in the allergen-exposed group (8). Activated eosinophils may be linked to the pathophysiology of asthma through the release of basic proteins and oxygen-derived free radicals (19). Microvascular leakage of plasma protein is an essential component of the inflammatory response in asthma and many of the inflammatory mediators implicated in asthma produce this leakage (20–22). The strong correlations observed here are consistent with the MRI signal reflecting eosinophil infiltration and activation, as well as leakage of plasma protein. It bears emphasis that the intensity and diffuse appearance of the MRI signals in the lungs after allergen challenge are qualitatively analogous to that of vasogenic edema at the infarct site reported in models of cerebral infarction in rodents, where edema is detected within a few hours until approximately four days after occlusion of the middle cerebral artery (23, 24).

The MRI signal also correlated highly significantly with the anti-inflammatory response to budesonide as determined by BAL fluid analysis. Rats pretreated with budesonide showed significantly less pulmonary edema and a significant decrease in inflammatory cell numbers, EPO and MPO activities following challenge with OA. Thus, a reduction in edema volume in actively sensitized, OA-challenged rats indicates that allergen-induced airway inflammation is inhibited by the anti-inflammatory drug. The MRI method could, therefore, be of importance for the non-invasive profiling of anti-inflammatory drugs in animal models of asthma and in the clinic.

Recently we have shown that the signal detected by MRI in the lungs of BN rats following i.t. administration of LPS had two components: one, of diffuse appearance and high intensity, was particularly prominent up to 48 h following LPS; the second, showing an irregular appearance and weaker intensity, was predominant at later time points, up to 16 days after LPS. Evidence that the latter is due to secreted mucus has been presented (16). The present analysis shows that the MRI signals in the lungs induced by LPS correlated only with the mucus concentration in the BAL. The fact that the MRI signals did not correlate with other parameters of inflammation could indicate that additional mechanisms are implicated in driving the mucus hypersecretion. Neuronal control of surface epithelial goblet cells regulates directly mucus secretion in rodents. Moreover, inflammatory mediators can act on epithelial goblet cells either directly, or indirectly via neuronal mechanisms to influence goblet-cell mucus secretion (5). It is possible that sustained neuronal activation, which would not be detected in our experimental approach, could be a major factor contributing to the mucus hypersecretion.

In conclusion, edematous signals detected by MRI in the lungs of actively sensitized rats after an i.t. instillation of ovalbumin correlated highly significantly with the protein concentrations, the eosinophil numbers and EPO activity determined by BAL fluid analysis. Thus, instead of BAL fluid analysis, which requires sacrificing the animals at each time point, following non-invasively the pulmonary edema formation by MRI provides a reliable means for assessing pulmonary inflammation after an allergen challenge. This approach has the clear advantage that, besides reducing considerably the number of animals used in the experiments, each animal can serve as its own control, since repeated measurements are feasible. Also, the response to multiple allergen challenge can be studied in the same animals (25). The MRI signal correlates with the anti-inflammatory response to budesonide, indicating that MRI provides a non-invasive means of monitoring the course of the inflammatory response and should prove invaluable in profiling anti-inflammatory drugs *in vivo* (26). Finally, after endotoxin challenge, mucus was the sole parameter in the BAL fluid that correlated significantly with the long lasting signal detected by MRI in the rat lung. The prospect of using MRI to detect non-invasively a sustained mucus hypersecretory phenotype in the lungs brings an important new perspective to models of chronic obstructive pulmonary diseases in animals.

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