The consequences of chronic allergic inflammation

Peter D Paré, Tony R Bai

Roger Altounyan had allergic asthma which was first diagnosed while he was a medical student. This disability allowed him to make the important discovery for which he is justly honoured by an annual lecture. His asthma is also the likely cause of the progressive respiratory failure that he suffered in his latter years. Shortly after he joined Fisons in 1956 Roger noted that one compound derived from khellin – a plant extract – appeared to decrease the antigen response in sensitised guinea pig ileum. This observation convinced him that it would be possible to develop a drug that treated asthma by blocking the release of mast cell mediators rather than by simply antagonising their effects. Between 1957 and 1964 he tested various compounds on himself for their anti-allergic properties. He was sensitive to grass pollen and guinea pig dander and he used himself as an assay system and nebulised numerous chemical modifications of khellin in the search for one that would attenuate his acute and delayed bronchoconstriction. In 1964 after testing hundreds of derivatives of khellin he identified a compound, disodium cromoglycate (Intal), that was consistently able to attenuate his allergic response.

At this point, after seven years of repeated antigen challenge, Roger's best forced expiratory volume in one second (FEV₁) was 1.5 litres. We know this because, in the pivotal paper published in 1965¹ demonstrating the effects of Intal, the baseline FEV₁ of the "typical subject" is approximately 1.5 litres. Antigen challenge of this typical subject caused a substantial fall in FEV₁ and Intal provided significant protection. The typical subject was, of course, Roger Altounyan himself. To prove that the drug had a beneficial effect in asthma a clinical trial was necessary.² This was conducted with Dr Jack Howell, his friend and collaborator, at Manchester University. A total of 10 subjects were studied using a double blind, crossover design. There was a two week run in period and two weeks of placebo, preceded or followed by two weeks of Intal. The outcome variable was the clinical assessment of patient status as judged by Howell. The results were unequivocal. In a blind fashion Howell judged that all 10 subjects were better during the treatment period with the active drug than with placebo. The rest is history. Intal became a major therapeutic agent in the

treatment of asthma and innumerable asthmatic subjects have derived clinical benefit from its use. The remarkable thing about Intal is that it is a drug with undoubted beneficial effect which acts at the very basis of asthma – the mediator release associated with acute antigen exposure – and yet it has virtually no detrimental side effects. In retrospect we can appreciate that Dr Altounyan was extremely lucky as well as perceptive and persistent.

It is of interest to contrast this remarkable story of the development of an important asthma medication with the process for bringing a new asthma medication onto the market in the 1990s. To develop a marketable drug a pharmaceutical company may begin with over 10 000 compounds of potential interest. It requires, on average, 12 years of exhaustive screening, toxicology studies, and clinical investigation before one drug reaches the market and the process costs millions of pounds. Roger Altounyan personally designed and brought to market a major new drug on a budget which was minuscule compared with that currently being used to develop new drugs – and in half the time!

Although Intal has no side effects the process of developing it did have one side effect – at least in Roger's case. He continued to test drugs on himself and later identified the drug nedocromil in a similar manner. In all he challenged himself at least 2000 times with antigen, developing acute and often late allergic responses. He required oral steroids in addition to lifelong Intal and β adrenergic agonists for the treatment of his asthma. By 1977 his FEV₁ had decreased to 0.7 litres. In his later years he developed cor pulmonale and required continuous oxygen. Although Roger smoked a pipe, he never smoked cigarettes.

What did Roger Altounyan's airways look like and why did he develop fixed airways obstruction? It is our premise that chronic allergic inflammation causes structural as well as functional changes in the airways; these changes not only contribute to the airway hyperresponsiveness which is a characteristic feature of asthma, but can also cause progressive fixed narrowing of the airways. Ideally we would have liked to show you Roger's airways; he would have enjoyed the prospect of his airway pathology enlightening others. However, we have examined the airways of chronic

Respiratory Health
Network of Centres of
Excellence, University
of British Columbia
Pulmonary Research
Laboratory, St Paul's
Hospital, Vancouver,
British Columbia,
Canada V6Z 1Y6
P D Paré
T R Bai

Reprint requests to:
Professor P D Paré.

asthmatic patients such as Roger and we are convinced that, despite its partially reversible nature, structural changes do occur in the airways of asthmatic subjects caused by chronic allergic inflammation and the repair process which follows episodes of acute allergic inflammation.

Roger challenged himself with guinea pig dander. In a recent study we have examined the effects of chronic antigen exposure on guinea pig airways. To make the symmetry perfect we should have used human dandruff as the antigen but we could not find a reliable source and had to use ovalbumin!

We examined airway structure and function in two groups of guinea pigs.^{3,4} One group received six weeks of biweekly exposure to an aerosol of ovalbumin after an initial sensitisation protocol. The second group received biweekly inhalation of an aerosol of saline and served as the control group. To determine if there was cellular proliferation in the airway wall we used the immunolocalisable tracer bromodeoxyuridine (BrdU) which is incorporated into replicating DNA (dividing cells) and can be detected using a monoclonal antibody. BrdU was injected intraperitoneally before each antigen or saline challenge. Measurements included: (1) *in vivo* dose-response curves generated by determining changes in pulmonary resistance in response to increasing doses of acetylcholine. We calculated PC₁₀ – the concentration causing a 10 times increase in resistance – and the maximal resistance that was achieved at the highest dose (RLmax); (2) *in vitro* dose-response curves generated by measuring the contractile force developed by tracheal muscle strips from these guinea pigs in response to the cholinergic agonist acetylcholine. The force generated by the individual strips of muscle was expressed as stress by dividing the force by the cross sectional area of the smooth muscle in the preparation; (3) on completion of the study we fixed the lungs and made morphometric measurements to look for structural alterations in the airway wall.⁵ All airways that were seen in cross section on histological examination were used to quantify

Figure 1 Schematic diagram of an airway in cross section showing the airway wall compartments which were measured in both the guinea pig and human lungs. Pi and Ai = inner perimeter and area, Pbm and Abm = basement membrane perimeter and area, Pmo and Amo = outer muscle perimeter and area, Po and Ao = outer perimeter and area, rmo = outer muscle radius.

Figure 2 Mean *in vivo* dose-response relationships of RL versus log dose of acetylcholine in control (○) animals and those exposed to ovalbumin (●). The PC₁₀ was lower and the maximal RL was greater in animals exposed to ovalbumin.

the dimensions of the various airway wall components (fig 1). We used the length of the airway internal perimeter (Pi) as our marker of airway size. The beauty of this measurement is that, even if an airway is constricted in the post mortem lung specimen, one can estimate the unconstricted airway size because the airways do not narrow concentrically; instead the airway mucosal layer folds and the length of the luminal perimeter remains constant. Thus, one can use Pi as a marker of airway size. We also traced the perimeter around the outermost layer of smooth muscle (Pmo) and the adventitial perimeter around the outermost layer of the airway at the parenchymal boundary (Po). We divided the airway into an inner wall internal to the smooth muscle and an outer wall external to the smooth muscle and also measured the smooth muscle area. The number of inflammatory and tissue cells in each compartment were point counted and the number of inflammatory and tissue cells which were positively stained for BrdU were similarly counted and expressed as a percentage of the total nuclei of each cell type (proliferation index = number of BrdU positive smooth muscle nuclei/total smooth muscle nuclei × 100).

The results of the *in vivo* dose-response curves are shown in fig 2. The average dose-

Figure 3 Mean *in vitro* trachealis muscle dose-response curves of muscle stress versus acetylcholine in control (○) animals and those exposed to ovalbumin (●). The maximal stress was significantly higher in animals exposed to ovalbumin.

Figure 4 Mean $\pm$ 95% confidence intervals of the square root of outer wall area versus P_i in control animals and those exposed to ovalbumin (OA). The outer wall was significantly thicker in the animals who were chronically exposed to antigen.

Figure 5 (A) Photomicrograph of a membranous airway from a control animal. The thickness of the outer wall is indicated by the arrows. (B) A similar airway in an animal exposed to ovalbumin showing the thickening of the outer adventitial layer. Bars = 30 μ m.

response curve of the animals treated with ovalbumin is shifted to the left and the calculated values of PC_{10} and RL_{max} were significantly different in the two groups (PC_{10} =

3.9 and 13.5 mg/ml in ovalbumin and control animals, respectively, and RL_{max} = 6.6 (0.4) and 3.4 (0.3) cm H_2O /ml/s in ovalbumin and control animals, respectively) ($p < 0.05$ for both).

The in vivo airway hyperresponsiveness was accompanied by an increased contractility of the trachealis muscle in vitro. Figure 3 shows the mean acetylcholine dose-response curves in the two groups. The maximal stress achieved by the trachealis preparations in ovalbumin-exposed animals was twice that of the control group (0.87 versus 0.39 kg/cm²). Could an increase in the force-generating ability of smooth muscle increase in vivo responsiveness? If the increased force generation was limited to the smooth muscle in the trachea it would have little effect since the trachea makes up only a small fraction of total airways resistance; however, if there was a more widespread increase in airway smooth muscle contractility it could play an important part in causing exaggerated in vivo airway narrowing. Although airway smooth muscle is capable of shortening to a remarkable extent (60–80%),⁶ it shortens much less in situ because the loads applied to the muscle by the surrounding airway wall components and the elastic recoil of the lung parenchyma are sufficient to limit the shortening considerably. If there is increased contractility of the muscles the same loads will be less effective and the smooth muscle will shorten more for the same stimulus.

Next we measured the dimensions of the airway wall compartments. Since airway dimensions can vary as a function of airway size, and since slightly different numbers and sizes of airways were sampled in the different groups, we compared airway dimensions by developing a relationship between airway size, as measured by P_i , and the areas occupied by the inner wall, the outer wall, and the smooth muscle.⁷ There was no difference between the groups in the area occupied by the inner wall or the muscle, but in the ovalbumin group the outer airway wall area was significantly increased (figs 4, 5A and B). Our morphometric analysis did not allow us to identify the nature of the thickening of this adventitial layer; it could be related to oedema or to increased connective tissue deposition as a consequence of the allergic inflammation. There was clear evidence of inflammation of the airways in the ovalbumin group. The inflammatory cells were almost exclusively eosinophils, as has been reported previously in this model.⁸ In animals exposed to ovalbumin there was an increased area fraction of inflammatory cell nuclei in the epithelium, the inner airway wall, and the outer airway wall. There were no inflammatory cells localised in the smooth muscle layer.

There was a significant increase in BrdU stained smooth muscle nuclei in the animals exposed to ovalbumin (23.0 (3.7)% versus 2.7 (1.1)%). There was also an increase in the proliferation index for epithelial cells (16.0 (3.3)% versus 4.8 (0.8)%) and a large increase in the number of BrdU stained inflammatory cell nuclei in the epithelium and adventitia. These results indicate that a relatively short

course of antigen exposure can cause inflammatory changes as well as the beginning of structural remodelling of the airway wall. There was no increase in smooth muscle mass despite a ninefold increase in the number of BrdU stained nuclei in the muscle. The incorporation of BrdU means that there has been DNA replication. It is conceivable that the increased incorporation in the animals exposed to ovalbumin was not due to cell division but is, instead, a reflection of repair to damaged DNA or DNA replication in the absence of mitosis (polyploidy). Alternatively, the result may mean that there has been smooth muscle cell division and proliferation with a decrease in the average smooth muscle cell size. More prolonged antigen exposure might be necessary to observe an increase in smooth muscle cell mass. Martin and colleagues have seen both an increase in smooth muscle mass and increased BrdU incorporation in brown Norway rats chronically exposed to antigen.⁹

We believe that these functional and structural changes in the airways of animals chronically exposed to antigen are the first stages in a remodelling process which causes substantial alteration in the airways of allergic asthmatic subjects. Recently Kuwano *et al* used similar morphometric techniques to examine the struc-

tural changes in the airways of chronic asthmatic patients.¹⁰ They studied airways from the lungs of 15 asthmatic subjects and compared them with the airways of 15 normal subjects. In eight of the asthmatic subjects the lungs were obtained at necropsy from patients who died of asthma – the fatal asthma group. The lungs from the other seven subjects came from a variety of sources: three were from asthmatic individuals who had died suddenly but not of asthma, and four of the lung specimens were from lobes obtained at the time of surgical resection for a lung lesion unrelated to asthma – the stable asthmatic group. The control group comprised 15 individuals who did not have airways obstruction or a history of asthma who were having surgical resection of a lung or lobe for a peripheral lung lesion. The same measurements that were made in the guinea pig airways were made on all airways in the human lungs that were cut in cross section. All three airway wall compartments were significantly increased in the asthmatic subjects compared with the control subjects, and the fatal asthmatic group had significantly thicker airway walls than the stable asthmatic group (table). The area occupied by the inner wall, outer wall, and muscle was 2–3 times greater in the asthmatic subjects.

How can these structural changes affect function? Increased inner wall area can amplify the effects of a given amount of airway smooth muscle shortening. Increased outer wall area can decrease the elastic load that the surrounding lung parenchyma provides to the smooth muscle by insulating the muscle from the elastic recoil of the lung, and an increase in the smooth muscle area, if it is associated with a concomitant increase in the force-generating ability of the muscle, will allow the muscle to shorten more for any elastic load. The mechanical effects of increased mucosal membrane thickness were first reported by Benson in 1975.¹¹ He showed that mucosal membrane thickening not only narrows the airway lumen but can substantially exaggerate the luminal narrowing caused by a given amount of smooth muscle shortening. An increase in the area of the outer airway wall can also enhance airway narrowing by increasing the amount of smooth muscle shortening. When smooth muscle contracts to narrow the airway it has to generate force to deform the surrounding lung parenchyma. The alveolar walls that attach to the outside of the airway contain elastic tissue which resists deformation and provides an elastic load for the muscle. If the outer or “adventitial” compartment of the wall area is increased in thickness, this decreases the interaction between the parenchyma and the muscle. An accumulation of oedema or connective tissue in this space allows the surrounding parenchyma to relax and a greater amount of smooth muscle shortening is then possible before the “springs” which represent the parenchyma are stretched to a sufficient extent to prevent further smooth muscle shortening (fig 6).

An increase in muscle mass has a similar effect. As the smooth muscle contracts it pulls

Airway wall dimensions: slope of the square root of airway wall area (mm²) versus Pi (mm)

	Fatal asthma group	Stable asthma group	Controls
Inner wall area	0.130**	0.102*	0.057
Outer wall area	0.167**	0.116*	0.079
Smooth muscle area	0.068**	0.044*	0.022

** Significantly different from stable asthma group and controls.
* Significantly different from controls.

Figure 6 Schematic drawing of an intraparenchymal airway. (A) Control airway: the springs represent the surrounding lung parenchyma which provides an outward dilating force. The resistance (R) of this airway has been arbitrarily designated 1. If the airway smooth muscle (ASM) of this airway shortens by 35% there will be a decrease in lumen area and an increase in airway resistance (R=8). (B) If the outer airway wall area thickens the springs are relaxed and the same smooth muscle stimulation causes greater smooth muscle shortening and a higher airway resistance (R=50).

the springs which represent the surrounding parenchyma. Smooth muscle shortening stops when the tension in the muscle is equal and opposite to the tension in the springs. If there is more muscle or stronger muscle there will be more shortening before the two tensions balance, and therefore there is more airway narrowing for a given stimulus.

In an attempt to define the relative importance of these structural changes we collaborated with Dr Rodney Lambert to develop a computer model of the tracheobronchial tree in which to examine simulated dose-response curves.¹² The morphometric data on airway wall dimensions for each generation of airways from the asthmatic and control subjects were entered into the model and the muscle in each generation was allowed to shorten in a dose-response fashion until the tension in the muscle was the maximal achievable at that length, given the load provided by the surrounding parenchyma. A number of assumptions were made in the model including that the increased muscle is accompanied by a parallel increase in force generating potential, that the airway smooth muscle is orientated purely circumferentially around the airway lumen, and that the elastic properties of the parenchyma are the same in asthmatic and control subjects. In fact it is possible that the increased airway wall thickness may stiffen the airway as has been recently suggested¹³ and could serve to attenuate excessive airway narrowing.

The results of these simulations showed that the asthmatic dose-response curve is markedly different from that of the controls. There was a shift to the left in the curve and a dramatic increase in the maximal airway narrowing that occurred. We computed the model using the airway geometry of the stable and fatal asthmatic groups separately and, although the curves in the stable asthmatic group were less dramatic, there was still a profound difference between the stable asthmatic group and the controls. To determine which of the three wall components was the most important cause of the altered function we then computed the model using the geometry of the controls but substituting, in turn, the asthmatic inner, outer, and smooth muscle thickening. Increasing the amount of smooth muscle had the most important effect on the curve. This analysis therefore suggests that, of the structural changes that occur in the airways in chronic allergic inflammation, it is the proliferation and/or hypertrophy of the muscle that is the most important.

In summary, we have tried to show what Roger Altounyan's airways looked like. We can

learn by looking back at Roger and his life. We can learn the value of persistence and single mindedness, and appreciate what a single person, determined to achieve something, can accomplish. I think we can also learn that chronic antigen exposure and the episodic allergic inflammation and repair it causes can have important consequences for the structure and function of the airways. We believe that Roger would have wanted us to learn from his pulmonary disease. The message that we should take from it is that only by interfering at a fundamental level with allergic inflammation can we ultimately hope to treat and cure asthma. Although bronchodilators relieve the acute constriction and corticosteroids non-specifically attenuate the inflammatory response, it is only by blocking excessive IgE production or the release of mediators and cytokines from inflammatory cells that we can prevent the consequences of chronic allergic inflammation. Roger recognised this and his drugs, although imperfect, come closest to the kind of agent that we need to develop.

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