EXPERIMENTAL AIRWAY HYPERSENSITIVITY IN SHEEP: A MODEL FOR ASTHMA

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ABSTRACT

This study aimed to establish an animal model for human bronchial asthma using locally bred Romney sheep. It was then planned to determine whether or not morphological and inflammatory factors in the ovine respiratory tract are associated with a predisposition to allergic bronchial hypersensitivity induced by inhaled *Ascaris suum* antigen.

The skin and airway responses to a commercial *A. suum* antigen were tested in adult Romney sheep from two local farms with and without previous exposure to pigs. Ninety percent of 101 adult sheep tested showed an immediate skin reaction, and about 70% of 43 adult sheep with positive skin reactions showed an immediate airway response, reflected as a significant increase in airway resistance and/or decreased dynamic lung compliance. Among these 43 sheep, 21 showed changes in both airway resistance and dynamic lung compliance (Group A); ten only in dynamic lung compliance (Group B) and 12 were non-responders (Group C). No significant changes were recorded when the same animals were given an aerosol of phosphate buffered saline. Although the sheep with previous exposure to pigs showed significantly greater skin reactions than those without exposure to pigs, they showed no significant differences in airway response to antigen inhalation. In addition, there was no correlation between the degree of skin reaction and the magnitude of bronchoconstriction.

Since no information was available on the respiratory tract-associated lymphoid tissue and cells in healthy sheep, study of this tissue and its associated epithelium was a prerequisite for studies of the morphological and inflammatory mechanisms involved in the development of allergic airway hypersensitivity. The ovine respiratory tract has five forms of lymphoid tissue; intra-luminal, intraepithelial, scattered forms, and dense and nodular aggregations; the dense and nodular aggregations being confined to the pharyngeal tonsil and bronchioles. Morphologically well-developed lymphoepithelium (M cells) is present only in the pharyngeal tonsil region, and absent in the lower respiratory tract. The M cell of the ovine pharyngeal tonsil is ultrastructurally and functionally similar to that in other mucosal tissues of this and other species, but its development and maturation takes place earlier than the bronchus-associated lymphoid tissue.

Mast cells in the lower respiratory tract of normal sheep are morphologically heterogeneous, and both formalin-sensitive and formalin-resistant types can be identified. The morphological and histochemical features of formalin-sensitive mast cells are similar to those from the human respiratory tract in several respects which enhances the use of the sheep model in the study of human allergic respiratory disease.

A morphometric comparison of airway structure and inflammatory components was conducted between the three groups of sheep with varying airway hypersensitivity. The epithelium of the small airways was significantly thinner and contained fewer goblet cells in the hypersensitive sheep (Groups A and B) than in non-reacting sheep (Group C). Mast cells from the hypersensitive sheep had a significantly greater volume density of secretory granules than those from non-reacting sheep. However, no morphological difference was found in the epithelial integrity of airways between hypersensitive and non-reacting sheep, and the permeability of tracheobronchial epithelium to horseradish peroxidase was of the same order in all groups. Similarly, the airway wall was not significantly thicker in hypersensitive sheep than in non-reacting sheep, and the shortening of smooth muscle required to cause complete airway closure was similar. The numerical density of mast cells, eosinophils, neutrophils and lymphocytes in the airways and lung was not significantly different between the groups.

These observations indicate that the *Ascaris*-induced airway response seen in Romney sheep is similar in several respects to that seen in human asthmatics and these sheep can therefore be used as an animal model to study human asthma. The current findings suggest that the presence of relatively low goblet cell density, thin epithelium, and high volume density of mast cell secretory granules in the small airways and lung may be important inherent factors responsible for the development of airway hypersensitivity in these sheep. It is concluded that most of the other morphological features observed in asthmatics and animal models are likely to be the result of allergic airway reactions rather than a fundamental difference between potentially allergic and non-allergic subjects.

STATEMENT

This is to certify that the work on which this thesis is based was carried out by the undersigned, and has not been accepted in whole or in part for any other degree or diploma. Assistance received is specifically recorded in the Acknowledgements section bound with this thesis.

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LIST OF PUBLICATIONS

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ABBREVIATIONS*

AB/PAS	alcian blue/periodic acid-Schiff
Ae	external area
Ae_r	external area in "relaxed" state
Ai	internal area
Ai_r	internal area in "relaxed" state
AM	alveolar macrophages
BAL	bronchoalveolar lavage
BALT	bronchus-associated lymphoid tissue
BSM	bronchial smooth muscle
C3a, C4a and C5a	complement C3a, C4a and C5a
Cdyn	dynamic lung compliance
CTMC	connective tissue mast cells
DAB	3,3 diaminobenzidine tetrahydrochloride
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
EPO	eosinophil peroxidase
f	respiratory frequency
FA	10% neutral buffered formalin
FAE	follicle-associated lymphoepithelium
GALT	gut-associated lymphoid tissue
HE	haematoxylin and eosin
HE/AB	haematoxylin and eosin/alcian blue
HRP	horseradish peroxidase
IELC	intraepithelial lymphoid cells
IFAA	isotonic formal-acetic-acid
Ig	immunoglobulin(s)
L	the length of outer layer of smooth muscle in airways
LAR	late airway response/reaction
LRT	lower respiratory tract
LT	leukotrienes
MBP	major basic protein
MC	mast cells
MMC	mucosal mast cells
NAHR	non-specific airway hyperresponsiveness
Nv	numerical density
PAF	platelet-activating factor

PAS	periodic acid-Schiff
PBS	phosphate buffered saline
Pe	external perimeter
PG	prostaglandins
Pi	internal perimeter
PMP	proportion of external perimeter of the airway occupied by muscle
PMS	the degree of muscle shortening
Ppl	intrapleural pressure
Pw	relative airway area
Raw	airway resistance
RNase	ribonucleic acidase
RTALT	respiratory tract-associated lymphoid tissue
SEM	scanning electron microscopy
SRS-A	slow-reacting substances of anaphylaxis
TEM	transmission electron microscopy
V	airflow rate
V_T	tidal volume
WA	wall area

* Abbreviations used in tables and figures are not included in this list.

INTRODUCTION

Asthma is a common respiratory disease of human beings characterised by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by widespread narrowing of the airways which is totally or partially reversible either spontaneously or by appropriate treatment (Daniele, 1980; Borish, 1987; Dail, 1988; Magunssen and Nowak, 1989). Clinically, the disease is manifested by paroxysms of cough, dyspnoea and wheezing with excess sputum production (McFadden and Ingram, 1980a; Schellenberg, 1985). Physiologically, the disease is characterised by an increase of airway resistance, total lung capacity and residual volume, a decrease of lung specific conductance, airflow rates and forced expiratory volumes, pulmonary hyperinflation and an imbalance of ventilation and perfusion (McFadden and Ingram, 1980b; Tattersfield and McNicol, 1987). The pathological changes of asthma extend to all bronchi and bronchioles down to 1 mm in diameter, and feature plugging of the airway lumen with exudate, epithelial shedding, squamous metaplasia, increase in numbers of goblet cells, thickening of the mucosal basement membrane, enlargement of bronchial mucous glands and smooth muscle, vasodilatation and oedema of the airway wall, and infiltration of the mucosa and submucosa by inflammatory cells, particularly eosinophils (Spencer, 1977; Dunnill, 1982).

The prevalence of asthma varies considerably between countries (Tattersfield and McNicol, 1987). In developed countries, there is an upward trend in the mortality and hospital admission rates for childhood asthma (Mitchell, 1985; Jackson *et al.*, 1988). Its prevalence has been estimated to be of the order of 3-5% of the population in the United States, and 3-12% in Britain (Tattersfield and McNicol, 1987; Drazen *et al.*, 1987). In New Zealand, about 27% of children suffer from asthma at some time before age 9 (Jones and Sears, 1987), and the hospital admission rates and mortality in childhood asthma are higher than in most other countries (Jackson *et al.*, 1988; Sears, 1988; Mitchell *et al.*, 1990). In 1985, there were 11,038 admissions to hospital for asthma with a mean length of stay of 4.6 days, the cost of which was about 17.3 million New Zealand dollars (Mitchell, 1989).

It is difficult to properly classify the clinical types of asthma because of an incomplete understanding of its pathogenesis. The disease has been divided into two subtypes: extrinsic (allergic) asthma and intrinsic (non-allergic) asthma (Daniele, 1980; Dunnill, 1982). The allergic type is of the most common, accounting for 25-30% of all cases of asthma and probably contributing to another third (McFadden and Ingram, 1980a), and is believed to be mediated by type I hypersensitivity. This type of asthma is generally associated with a personal and/or family history of atopy, positive skin reaction, raised serum immunoglobulin E levels and positive bronchial provocation tests (McFadden and Ingram, 1980a). The disease begins at

any age, but about half of the cases develop before age 10. Most acute attacks of allergic asthma tend to be short-lived, following which the patients can clinically recover completely, although sometimes severe airway obstruction persists for days or weeks as status asthmaticus (McFadden and Ingram, 1980a).

The airway reaction which occurs in asthmatics following bronchial provocation may be one of three types: an isolated early asthmatic reaction, an early followed by a late asthmatic reaction, and an isolated late asthmatic reaction (Tattersfield and McNicol, 1987). The early asthmatic reaction usually develops within 10 minutes of provocation by inhalation, reaches its maximum within 30 minutes and is generally resolved within 1-3 hours. The late reaction usually starts after 3-4 hours, reaches its maximum over the next few hours and clears within 24 hours or more (O'Byrne *et al.*, 1987).

The pathogenesis of allergic asthma has yet to be fully clarified. Episodes of asthma can be evoked by many stimuli, such as infections, exercise, antigens, occupational stimuli, environmental causes, pharmacological stimuli and emotional stress (McFadden, 1984). Several theories have been proposed to explain the development of airway hypersensitivity and allergic asthma. These include the type I hypersensitive reaction, the β-adrenergic blockade theory, the inherent twitch of bronchial smooth muscle, and neurogenic mechanisms (Daniele, 1980; Tattersfield and McNicol, 1987). Recent studies have favoured the concept that asthma is a chronic inflammatory disease involving the interaction of many inflammatory cells (Hogg, 1982; Borish, 1987; Kay, 1987; Barnes, 1989).

Investigation of basic mechanisms in the pathogenesis of asthma has been hampered by difficulties in gaining direct access to human asthmatic airways because of ethical and safety reasons. Although the use of fibre-optic bronchoscope in asthmatics has widened the possibilities of studying asthma directly in volunteers (Flint *et al.*, 1985a,b), results from such studies have been usually compromised by a smoking history and low grade of other inflammatory diseases in the patients. Recent studies have also shown that there are great variations in the inflammatory cell counts between bronchial biopsy specimens (Azzawi *et al.*, 1990). Alternative animal models are, therefore, necessary to advance our understanding of the pathogenesis of this disease.

Several animal models, including rats, guinea pigs, dogs, monkeys, rabbits and sheep, have been used to study the pathogenesis of airway hypersensitivity and allergic asthma (Booth *et al.*, 1970; Patterson and Kelly, 1974; Hogg *et al.*, 1979; Wanner *et al.*, 1979; Kallos and Kallos, 1984; Hamel *et al.*, 1986; Murphy *et al.*, 1986; Eidelman *et al.*, 1988). Among these species, the sheep is considered to be one of the most satisfactory models (Wanner and

Abraham, 1982). Most sheep tested overseas have a natural skin reaction and an immediate airway response to *Ascaris suum* extract (Wanner *et al.*, 1979; Bosse *et al.*, 1987; Okayama *et al.*, 1989). The airway response in this species is similar in both physiological and pharmacological aspects to that seen in human asthmatics, and is more consistent than the antigen-induced bronchoconstriction in dogs and guinea pigs (Wanner and Abraham, 1982). In addition, some allergic sheep also exhibit late airway reaction (Abraham *et al.*, 1983). Both the early and late airway reactions in sheep have been demonstrated to be antigen-specific and mast cell mediator-dependent (Wanner *et al.*, 1979; Abraham *et al.*, 1983; Okayama *et al.*, 1989). Over the past decade, the sheep has been increasingly used as a model of allergic airway hypersensitivity (Wanner *et al.*, 1979; Kleeberger *et al.*, 1985; Bosse *et al.*, 1987; Okayama *et al.*, 1989). Investigations using this model have produced much useful information. However, almost all studies to date have focused on physiological and pharmacological aspects of the disease (Wanner and Abraham, 1982), and morphological and immunological studies on this model are relatively scant. Further information on these aspects will be useful for fully using this model and for a better understanding of the mechanism of the development of allergic airway hypersensitivity.

It is important to state here, that like most animals, the sheep is not a complete animal model of human asthma since sheep do not spontaneously show clinical symptoms of asthma. Also sheep do not exhibit pathological changes characteristic of asthma. However, the term "asthma model" will be used throughout this thesis since this term has already been used for the sheep model by several workers (Wanner and Abraham, 1982; Ahmed *et al.*, 1983).

In New Zealand, only very limited studies of airway hypersensitivity and asthma have been carried out using animals; the guinea pig being the main animal model available (Galland and Blackman, 1989).

Morphological studies of human asthma undertaken to date suffer from the disadvantage that most of the features described are likely to be associated with the end result of the disease process and it is not known which (if any) morphological features may predispose an individual to develop airway hypersensitivity. Recent studies by Hopp *et al.* (1990) have shown that enhanced airway reactivity usually precedes the development of asthma. It is therefore of interest to examine the possibility that certain morphological and cellular abnormalities may exist before the development of airway hypersensitivity.

The present study had two main aims. The first was to evaluate the suitability of locally bred Romney sheep as a model to study human asthma in New Zealand. Knowledge of the prevalence of natural responders in sheep in New Zealand would thus be available for future

workers in this country wishing to use the ovine model. The second aim was to use the sheep model established to determine whether or not morphological and inflammatory factors in the ovine respiratory tract could be associated with a predisposition to develop allergic airway hypersensitivity to inhaled *A. suum* antigen.