The effect of macrolides on allergic rhinitis versus chronic rhinosinusitis-

An in-vitro study

Romana Kuchai

Department of Otorhinolaryngology & Department of Academic Respiratory Medicine,

ABSTRACT

The effect of macrolides on allergic rhinitis versus chronic rhinosinusitis- An in-vitro study.

Background
The mechanisms of the rhinitic process are complex. Previous studies upon nasal epithelial cells have begun to investigate rhinitis. HNECs from turbinate explant tissue were taken from three patient groups (Normals, Chronic Rhinosinusitics and Rhinitics).

Aims
The study, firstly, aims to establish fundamental differences in cytokine activity between allergic rhinitis and chronic rhinosinusitis by analysing baseline levels of cytokines IL-6 and IL-8 and subsequent impact of bacterial endotoxin. Secondly the study analyses the affect of macrolides on activity in each sub-group.

Methods
HNECs were grown from the biopsy specimens as explant culture. Standardised exposures to LPS bacterial endotoxin and macrolide were carried out. The concentration of each mediator present in the medium at the end of incubation was assessed by ELISA). A final quantity of total cellular protein was obtained.
Results

Baseline levels of IL-6 in unstimulated Allergic Rhinitics are significantly higher than in Normal patients. Baseline levels of IL-8, however, are lowest in Allergics. LPS significantly stimulates Allergics to increase production of both IL-6 and IL-8. Macrolides lower IL-6 and IL-8 in both stimulated and unstimulated AR cells.

Baseline levels of IL-6 and IL-8 are higher in CRS than AR and Normals. LPS significantly raises IL-6 and IL-8 in CRS. Macrolides increase IL-6 and IL-8 in stimulated CRS cells however reduce levels of both in un-stimulated cells.

Discussion

Pre-existing neutrophilic and eosinophilic activity in CRS subjects may explain the increased baseline levels of both cytokines upon macrolide exposure.

Whilst some studies have suggested macrolides act as antimicrobial, others have suggested that it is their anti-inflammatory effects that are more relevant. Treatment for Allergic Rhinitis needs to be effective long-term. The results here are novel and encourage further research to improve understanding of the effects of macrolides in a potentially pivotal role.
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5. ABBREVIATIONS

ATP - adenosine tri-phosphate
AR - Allergic rhinitis
CAMs - cell adhesion molecules
CRS - Chronic rhinosinusitis
DNA - deoxyribonucleic acid
ECP - eosinophilic cationic protein
ELISA - enzyme-linked immunosorbent assay
FESS - Functional endoscopic sinus surgery
FEV1 - Forced expiratory volume in 1 second
FVC - Forced vital capacity
GM-CSF - granulocyte-macrophage colony stimulating factor
GTP-ases - guanine tri-phosphatase
HBEC - human bronchial epithelial cell
HNEC - human nasal cell epithelial cell
HRV - human rhinovirus
ICAM-1 - intracellular adhesion molecule-1
IFN - interferon
Ig - immunoglobulin
IL - interleukin
Mag - magnification
MCP-1 - monocyte chemotactic protein-1
MIP-1 - macrophage inhibitory protein-1
mRNA - messenger ribonucleic acid
N - Normal
NARES - non-allergic rhinitis eosinophilia syndrome
NFL - nasal lavage fluid
PAR - perennial allergic rhinitis
PAR2 - Protease activated receptors-2
PCR - polymerase chain reaction
PVC - polyvinyl chloride
RANTES - released on activation, normal; T cell expressed & secreted
RAST - radioallergosorbent test
SAR - seasonal allergic rhinitis
sCAMs - soluble cell adhesion molecules
sICAM-1 - soluble intracellular adhesion molecule-1
SPSS - statistical package for social science
TGF-β - transforming growth factor β
Th-1 - type 1 T-helper
Th-2 - type 2 T-helper
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>tumour necrosis factor-α</td>
</tr>
<tr>
<td>URTI</td>
<td>upper respiratory tract infection</td>
</tr>
<tr>
<td>UV</td>
<td>ultra-violet</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular intercellular adhesion molecule-1</td>
</tr>
</tbody>
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I wish to dedicate this work to my husband Evangelos, two children, Sophia and Elyas and my beloved parents.

6.2 CONTRIBUTIONS

I confirm this work is a result of my own endeavour with specific contributions made by following:
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- Mr. R. Sapsford - experimental design and technical support
- Dr. G. Donaldson and Dr. J. Hurst - statistical analysis
- Allergy clinic at St. Bartholomew’s Hospital

6.3 PRESENTATIONS

Preliminary data has been presented at the Otorhinolaryngological Research Society Meeting, London, September 2008
7.0 INTRODUCTION
7.1 **NASAL ANATOMY AND PHYSIOLOGY**

The healthy adult, the nose is a vital organ in the respiratory pathway, acting as not only an air-conditioner for warming and saturating inspired air but also acting as a filter by removing particulate debris and infectious agents. It thereby allows conservation of heat and moisture from expired air. It is known that we breathe 10,000 to 20,000 litres of air per day, the majority of which passes through the nose.

7.1.1 **Embryology**

The development of the nose begins in the third intrauterine week. By the fourth week, paired depressions termed olfactory placodes become visible in the cranial ectoderm above the stomatodeum. The mesoderm around these olfactory placodes thickens to form the medial and lateral nasal folds. The nasal placodes deepen further to form nasal pits and then subsequently nasal sacs by the fifth week. The medial nasal folds fuse to become the upper lip, premaxilla and primitive nasal septum. With growth of the maxillary process of the first branchial arch and the mesoderm of the frontonasal process, the primitive palate is formed. The nasal sacs deepen and thin the bucconasal membrane until it ruptures to form the posterior choanae.

The nasal septum continues to form from contributions made by the frontonasal process and the forebrain capsule. The union of the palatal processes of the
maxillary processes form the nasal capsule, in mesoderm. Chondrification and subsequent ossification occurs.

7.1.2 **Gross Anatomy**

The nose is composed of a cartilaginous and bony framework. The basic structure consists of a floor, a roof and 2 lateral walls. The floor of the nose is formed by the hard palate with the cavity extending posteriorly to the soft palate. Here the choanae open into the nasopharynx. Anteriorly the roof of the nose is formed by the cranial fossa and contains the cribriform plate, a thin lamina of bone perforated by olfactory nerve fibres. The roof of the sphenoid arches downwards to form the superior aspect of the nasopharynx. The bony structure of the nose comprises of paired bones which meet in the midline to form a bony pyramid with the base of the pyramid being formed by the frontal process of the maxilla. The lower two thirds of the external framework of the nose are formed by the paired upper and lower lateral cartilages and intervening sesamoid cartilages.

The nasal septum divides the internal structure of the nose. This is again an osseo-cartilaginous structure. It is comprised mainly of the septal cartilage which has a free anterior border but articulates postero-superiorly with the perpendicular plate of the ethmoid, postero-inferiorly with the vomer and inferiorly with the maxillary crest. It receives additional contributions from the palatine bone and the anterior spine of the maxillary crest.
The nasal vestibule begins at the nasal alae and is bounded internally by the region of the nasal valve. This area has short thick hairs (vibrissae) that aid in filtration of gross particulate matter. The nasal valve region is formed by the junction of the upper lateral cartilages, the nasal septum and the inferior turbinate. As this area accounts for some 50% in airway resistance, changes in cross-sectional area at this site can contribute significantly to total airway resistance. Area changes at this site are dependent upon the tone of the nasal vestibule. The tone in this region is mediated by the dilator alae muscles i.e. dilator nasae and dilator aleque nasae. This is under neurogenic control facilitated by branches of the facial nerve and an increase in tone of these muscles (flaring of the nostrils) results in an increase in cross-sectional area and a concomitant decrease in airway resistance. However, an increased negative pressure in this region, such as during increased respiratory effort, can have an opposite effect by causing the nasal vestibule to collapse inwards and decrease cross-sectional area.

The lateral wall of the nasal cavity passes superiorly to meet the nasal septum and cribriform plate. It has three elongated processes, the nasal turbinates, which are scroll-shaped bony structures covered by highly vascular mucosa. Such vascularity enables these turbinates to change in size to facilitate their role in the physiological functions of the nose. Beneath each of these turbinates lie corresponding openings (ostia). The inferior ostium receives the naso-lacrimal duct, the superior ostium receives the drainage of the sphenoid and posterior ethmoid sinuses with the
middle meatus which is a complex of openings receiving the drainage of the remaining nasal sinus.

Fig.1

(Courtesy of www.blumdesign.com, Jan 2008)

7.1.3 Nasal Histology

The nasal cavity is lined by 3 types of epithelium: (Eccles, 1996)

1) Stratified squamous epithelium in the nasal vestibule and nasopharynx.

2) Pseudostratified ciliated columnar epithelium within the main respiratory area of nasal cavity and paranasal sinuses.
3) Specialised olfactory epithelium with ciliated receptor cells in the roof of the nose.

The nose lies at the junction of the upper respiratory tract with the external stratified squamous epithelium of skin and thus has a brief transitional zone i.e. the nasal vestibular area is lined by stratified squamous hair bearing skin but beyond this the mucosa is transitional and then only becomes the typical respiratory pseudostratified ciliated columnar epithelium. Indeed, while the mucosa of the inferior turbinate is entirely ciliated at birth, it is thought that as a result of airflow over its anterior extremity, its ciliated character is lost at this site.

Additionally, conforming to its role in olfaction, the area at the roof of the nasal cavity in the region of the cribriform plate contains a concentration of special olfactory cells. These cells also extend from here to the epithelium of the superior turbinate.

Fig.2 Nasal Epithelium
7.1.4 Nasal Cytology

The nasal epithelium has within it four cell types—namely basal, goblet, columnar and inflammatory cells.

Columnar cells account for the pseudostratified nature of respiratory epithelium as they are all anchored to the basement membrane. Their surface area is increased by microvilli which also help to prevent drying of the epithelium. Beyond the nasal vestibule and tip of the inferior turbinate, most columnar cells are also ciliated. The
breakdown of adenosine tri-phosphate (ATP) in the nine anterior micro tubules provides the energy for ciliary movement (Brain, 1989). Cilia provide an efficient transport of nasal mucus with co-ordinated rhythmical activity. Human and animal studies suggest that mucociliary clearance is influenced by the autonomic nervous system. Recent work seems to suggest that human nasal epithelium does play an immunomodulatory role in the upper airway by synthesis and thereby release of specific biochemicals, including pro-inflammatory cytokines and cell adhesion molecules.

In those with an enhanced inflammatory response e.g. allergic rhinitics, cells including eosinophils and mast cells proportionately increase. A ground substance and tissue fluid contain loose connective tissue, submucosal glands, vasculature and extra-vasculature immunocytes. This ground substance is a gel composed of water, electrolytes and serum proteins.
7.1.5 Nasal Glands

These types of glands can be found in the epithelium and submucosa of the nose: anterior serous glands, seromucous glands and intra-epithelial glands.

Tos deduced that anterior serous glands were few with 20-30 located on the septum and equal number on the lateral wall and were thereby their total secretion was minimal.

Seromucous glands lie deep within the lamina propria. Vessels, nerves and fibres form within them. The glands are thought to total more than 90,000.

Intraepithelial glands are located within the epithelium. They produce a small amount of mucus in relation to seromucous glands.
7.1.6 Nasal Vasculature and Lymphatics

The nasal submucosa is a very vascular layer.

The nose receives blood supply from both the internal via the ophthalmic artery which gives rise to the anterior and posterior ethmoid arteries. They supply the anterosuperior portion of the septum, the lateral nasal walls, the olfactory region and a small part of the posterosuperior region. The external carotid arteries supply the nose via the internal maxillary arteries which enters the nasal cavity through the sphenopalatine foramen just behind the posterior end of the middle turbinate. The sphenopalatine artery gives rise to posterior lateral and septal branches. These branches are the majority of the blood supply to the nose including the floor.

The venous supply accompanies the corresponding arteries and drains into the pterygoid plexus. This system also drains the ophthalmic plexus in the orbit and most importantly are part of the system draining into the cavernous sinus. There is a large anastomotic system within the veins of the face, palate and pharynx. It must be remembered that the system is a valveless one, thereby predisposing to the spread of infection.

Lymphatic system of the nose drains from the vestibule to the external nose however endonasally drainage leads posteriorly.

7.1.7 Submucosal immunocytes

Immunocytes are characteristically found in the nasal submucosa include eosinophils, T and B lymphocytes, mast cells, neutrophils, basophils and plasma
cells.

7.1.8 Nasal Nerve Supply

The nasal cavity is supplied by the nerve to the first branchial arch namely the trigeminal nerve. Fibres from the ophthalamic and maxillary branches of the trigeminal nerve provide tactile and sensory relays but also provide secretomotor fibres. Sympathetic innervation is provided by the superficial petrosal nerve and sympathetic nerve supply is provided by the deep petrosal nerve. These two nerves together form the nerve of the pterygoid canal (Vidian nerve).

7.1.9 Nasal Secretions

Nasal secretions enable an effective defense mechanism between a delicate respiratory mucosa and dry, polluted, cold air. The nose and paranasal sinuses produce approximately 1 litre of secretions each day. The two main constituents are a superficial gel and a deep periciliary layer.

7.1.10 Nasal Physiology

The nose whilst superficially being important in facial cosmesis, has several physiological roles. Namely-

1. Its functions as part of the gateway to the upper respiratory tract i.e.
   a. Provision of airway resistance with the nasal valve region contributing to 50% of airway resistance
   b. Humidification of air
c. Warming of air  
d. Filtration of air  
e. Guardian to foreign matter; both mechanical e.g. by vibrissae and mucous and immunological

2. Olfaction (and its associated contribution to the sensation flavour)  
3. Together with the paranasal sinuses, enhancement of the timbre of voice.

7.2 NASAL INFLAMMATION

The term ‘rhinitis’ implies inflammation. The symptoms include sneezing, irritation, anterior discharge, hyposmia, anosmia and nasal obstruction. Secondary symptoms include headache, facial pain, ear popping, nasal obstruction, dry throat, post-nasal drip, cough and eye symptoms. It is estimated that morbidity associated with nasal symptoms is responsible for over £1.5million caused by loss of working hours.

The pathophysiology of rhinitis, allergic and non-allergic, is yet to be fully understood. The classification of rhinitis is evolving. Allergic rhinitis whether, seasonal, perennial or episodic is easier to define, investigate and manage. Non-allergic rhinitis, however is a more difficult entity with a more aetiological basis of diagnosis. For the purposes of this thesis we focus on skin prick test positive allergic rhinitis and chronic rhinosinusitis confirmed by a pre-operative CT-scan, the criteria of which I will explain in further detail later.
7.2.1 Nasal Allergy

The nasal allergic response consists of two responses that overlap; a mast cell mediated immediate hypersensitivity reaction and a late phase response. The immediate response is seen classically in allergic rhinitis within 2-5 minutes of allergen exposure and reaches a peak after approximately 15 minutes. The late phase response occurs approximately 4-6 hours later and takes place under the influence of a vast number of inflammatory mediators such as cytokines released from nasal epithelial cells and immunocytes.

7.2.2 The immediate hypersensitivity response

Pathophysiology

The immediate response is a Gell & Coombs Type I response, mediated by mast cell degranulation. Cross-linking of mast cells with immunoglobulin E (IgE), triggers the process. An influx of intracellular calcium and exocytosis of granule content (Bousquet et al, 1996) is stimulated. A specific antigen usually will then cause cross-linking however mast cells can be triggered by other stimuli e.g cytokines, neuropeptides, complement.

7.2.3 IgE

IgE production occurs locally at mucosal surfaces, when an allergen enters the body. The allergen interacts with mucosal antigen presenting cells, which capture, process and present antigen to T-helper
cells, which capture, process and present the antigen to T helper lymphocytes. These T lymphocytes become activated and via interleukin-4 (IL-4), IL-6 and IL-13 signalling, cause B lymphocytes to preferentially produce IgE (Vercelli & Geha, 1993).

IgE is locally produced and first attaches to local immunocytes, but excess IgE enters the systemic circulation and binds to both circulating basophils and tissue fixed mast cells throughout the body. The normal function is IgE is as defence against parasitic infection, however the characteristic feature of atopy is inappropriate preferential production of IgE in response to antigenic stimulation by common aero-allergens.

7.2.4 Mast cells

Mast cells are superficially located in the respiratory epithelium and are ideally placed to respond to inspired antigens. The immunological staining of mast cells in nasal biopsies shows an increase in the number of these cells within the nasal epithelium in both perennial and seasonal rhinitis compared with non-atopic, non-rhinitic individuals (Bentley et al, 1992; Bradding et al, 1993).

7.2.5 Mast cell derived inflammatory mediators

Mast cell granules contain pre-formed mediators including histamine, heparin and proteolytics enzymes. These are responsible for the classic
nasal symptoms seen in the immediate hypersensitivity response—itch, sneeze, discharge and blockage—through interaction with receptors present on both neural and vascular elements within the nasal mucosa. Other pre-formed mediators include eosinophil chemotactic factor, neutrophil chemotactic membrane occurs to produce de novo synthesis of inflammatory mediators, such as leukotrienes, prostaglandins and platelet activating factors (Lee, Naclerio et al. 1994).

7.2.6 Mast cell pro-inflammatory Cytokines
Nasal mast cells also contain pre-formed cytokines, including IL-4, IL-5, IL-6 and Tumour necrosis factor-α (TNFα) which contribute to the immunocyte growth, differentiation, adhesion and activation seen in the late phase response (Bradding, Feather et al. 1993; Bradding, Okayama et al. 1995).

7.2.7 The late phase response
The immediate hypersensitivity response does not entirely explain the symptomatology of patients with allergic rhinitis. The following observations support this notion:

1) The duration of the early reaction to antigen is measured in minutes, whereas clinical disease is more prolonged, with patients complaining of symptoms hours after allergen exposure.
2) Biopsies of atopic nasal mucosa during the allergy season show an inflammatory cellular infiltrate, whereas studies of acute hypersensitivity reaction show only mast cell degranulation and tissues oedema.

3) Systemic steroids, although useful in refractory cases of allergic rhinitis, do not inhibit the early reaction.

The late phase response - Definition
The nasal phase response is defined as a recurrence of symptoms and the appearance of inflammatory mediators in nasal secretions 3-11 hours after allergen exposure. (Naclerio, 1999).

7.2.8 Pathophysiology
A significant increase occurs in the number of immunocytes in the nasal lining, including basophils, eosinophils, T-helper lymphocytes, neutrophils and mononuclear cells (Okuda, Sakaguchi et al. 1983; Okuda, Ohtsuka et al. 1985; Bascom, Wachs et al. 1988; Howarth 1995).

This response usually occurs within a few hours after the early response. This cellular influx is mediated by cytokines such as IL-1β, TNFα, IL-3, IL-5, IL-8, “released on activation, normal T cell expressed and secreted” (RANTES), granulocyte-macrophage colony stimulating factor (GM-CSF) and adhesion molecules such as intercellular adhesion
molecule-1 (ICAM-1) (Ciprandi, Pronzato et al. 1994), vascular intercellular
adhesion molecule-1 (VCAM-1), selectins and integrins (Bachert, Hauser et al.
1995).

7.2.9 Cellular infiltration- T lymphocytes

In contrast to the immediate hypersensitivity response, much of the nasal
late phase response is thought to be under the control of pro-inflammatory
cytokines. The two principal mucosal sources for the cytokines are nasal
respiratory epithelium and T helper lymphocytes. These lymphocytes
have been classified into subsets Th1 and Th2 on the basis of their
distinct cytokine profile. Th2 clones predominate in allergic rhinitis and
secrete TNFα, IL-3, IL-4, IL-5, IL-6, IL-10, IL13 GMCSF. Th2 clones
are more effective than Th1 at assisting nasal IgE production.

7.2.10 Nasal epithelial cell derived inflammatory mediators

Nasal epithelial cells hold two distinct roles in the inflammatory
response. They enable clearance of particulate matter from the airway and
also act as a physical barrier to the entry of noxious agents. Recent
studies have shown that nasal epithelial cells play a pivotal role in the
initiation and control of the inflammatory process. They are able to
release biologically active mediators which modulate the function of
other inflammatory cells implicated in the pathogenesis of rhinitis
(Cromwell, Hamid et al. 1992; Devalia, Campbell et al. 1993). These mediators include pro-inflammatory cytokines and cell adhesion molecules, nitric oxide and arachidonic acid metabolites. Many studies have identified structurally and functionally related inflammatory cytokines (Miller and Krangel 1992).

7.2.11 Pro-inflammatory cytokines

These mediators are of particular interest in allergic rhinitis as they influence the activity of immunocytes such as eosinophils, neutrophils, T-lymphocytes and mast cells. Their presence is a key feature of rhinitis. Studies in vivo and in vitro have shown that HNECs generate a wide variety of cytokines including IL1β, TNFα, IL-6, IL-5, IL-8, GM-CSF, RANTES AND MCP-1 (Devalia, Campbell et al. 1993; Kenney, Baker et al. 1994; Davies, Wang et al. 1995; Mullol, Xaubet et al. 1995; Mullol et al, 1995). However there is no conclusive evidence to date that HNECs release appreciable amounts of IL-3, IL-4 or IL-13.

The airway epithelial cell-derived cytokines can be divided into 4 groups according to their main functions:

1) Colony- stimulating factors, which promote the differentiation and survival of the recruited inflammatory cells

2) Growth factors, which regulate the growth and differentiation of airway epithelial cells themselves
3) Chemotactic factors which can influence the chemotaxis of the other inflammatory cells

4) Pro-inflammatory multifunctional cytokines, which can initiate and amplify events

7.2.12 Colony stimulating factors

Granulocyte-macrophage colony stimulating factor (GM-CSF) remains the main cytokine in this group. It serves with two important functions in the pathway. Firstly it has chemo-attractant properties as well as potentiating the differentiation and survival of eosinophils and neutrophils (Resnick and Weller 1993; Trigg, Manolitsas et al. 1994; Borish and Rosenwasser 1996; Humbert 1996) demonstrated a significant correlation between epitheial cell expression of GM-CSF and the number of activated eosinophils in atopic respiratory mucosa (Trigg, Manolitsas et al. 1994).

7.2.13 Growth factors

TGF-β is the most studied transforming growth factor. It is produced by airway epithelial cells and mediates cell growth and differentiation (Levine 1995; Borish and Rosenwasser 1996).

It also possesses anti-inflammatory activity in that it inhibits T helper lymphocytes and cytotoxic (CD-8) lymphocytes and lessens allergic inflammation by suppressing IL-4 induced IgE production by B
lymphocytes (Levine 1995).

7.2.14 Chemotactic factors

Chemotactic factors generated by HNECs include GM-CSF (discussed earlier), “released on activation, normal T cell expressed and secreted” (RANTES), monocytes chemotactic protein-1 (MCP-1) and IL-8, RANTES and MCP-1 both belong to the same chemokine subfamily. RANTES is a potent eosinophil chemotaxin, MCP-1 induces monocyte and basophil activation and chemotaxis (Schall 1991; Miller and Krangel 1992; Alam, Stafford et al. 1993).

IL-8 is a potent chemo-attractant for neutrophils and eosinophils (Baggiolini and Clark-Lewis 1992; Baggiolini, Boulay et al. 1993; Baggilolini and Dahinden 1994).

7.2.15 Multifunctional cytokines

IL-1β, TNF-α, IL-5 and IL-6 are multifunctional cytokines synthesized and released by airway epithelial cells. They all have pro-inflammatory effects on a variety of target cells (Devalia, Campbell et al. 1993; Levine 1995).

IL-1β and TNF-α are potent inducers of cell adhesion α molecules such as ICAM-1, V-CAM-1 and E-selectin, which facilitate the adhesion and migration of immunocytes (Montefort, Holgate et al. 1993; Howarth 1995; Howarth 1995; Howarth, Bradding et al. 1995; Howarth and Holmberg 1995).
Other studies indicate that TNFα activates T-lymphocytes, mast cells and eosinophils and is chemo-attractant for neutrophils and monocytes (Bradding, Feather et al. 1993; Bradding, Okayama et al. 1995; Bradding and Holgate 1999). Several studies have shown the release of inflammatory cytokines and adhesion molecules by human bronchial epithelial cells is influenced by bacterial induction. In their hypothesis, Khair et al, 1996, described the infiltration and activation of neutrophils as a result of increased release of pro-inflammatory mediators from respiratory epithelium. Bacterial products such as endotoxins are known to influence this activity.

IL6, IL8, TNF-alpha and ICAM-1 have been shown to develop enhanced expression as a result of bacterial endotoxins. Khair et al in 1995 studied the effects of erythromycin on the release of IL6, IL8 and sICAM-1 following haemophilus influenza endotoxin stimulation. Their results showed an increase in neutrophil chemotaxis within cultured human endothelial cells.
IL-5 plays an important role in the proliferation, activation and chemo-attraction of eosinophils as well as enhancing histamine and leukotriene release from basophils (Salvi, Semper et al. 1999). IL-6 has a role in the cell proliferation and IgE synthesis.
7.2.16 Cell adhesion molecules

Cell adhesion molecules are (CAMs) are specific cell surface receptors which mediate adhesion of cells to one another and to the extra-cellular matrix, (Albelda and Buck 1990; Mackay and Imhof 1993). As a group they are of importance in the maintenance if tissue architecture, the inflammatory response, tumour metastasis and wound healing.

7.2.17 Protease-activated receptors

Protease-activated receptors (PAR) are involved in the contribution of airway epithelial cells to the development of inflammation by release of pro- and anti-inflammatory mediators. Ostrowska et al evaluated the influence of LPS and continuous PAR activation on PAR expression level and the release of pro-inflammatory chemokine IL-8. The study was carried out on primary human small airway epithelial cells and two airway epithelial cell lines. LPS specifically up-regulated expression of PAR-2 agonists but not PAR-1. The authors also found PAR-2 but not PAR-1 caused production of IL-8 from epithelial cells. There was a potentiation of the stimulation of the IL-8 synthesis and release by PAR-2 in human lung epithelial cells. The study has confirmed an interaction between LPS and PAR agonists in affecting PAR regulation and IL-8 production.

The airway epithelium acts as a barrier between the environment and sub-epithelial tissues. Winter et al discussed the elements that impose restrictions within the
epithelium. The protease-activated receptor-2 (PAR2) receptor is expressed in airway epithelium and its activation initiates multiple effects including enhanced airway inflammation and reactivity. The study hypothesized that activation of PAR2 would interrupt E-caderhin adhesion and compromise the airway epithelial barrier. An immediate 50% decrease in trans-epithelial resistance of primary human airway epithelium persisted for 6-10 minutes. PAR-2 may therefore be involved in the pathophysiology of CRS at different sites of activation however the effects of protease-activated receptor-2 (PAR-2) stimulation on inflammation mechanisms of chronic rhinosinusitis (CRS) are still unknown.

7.2.18 Nasal Hyper-reactivity

Re-challenge with the allergen following an initial provocation results in an increase in inflammatory mediator release. It is suggestive of both mast cell and basophil activation. Most importantly, the dose of allergen necessary to induce a clinical reaction is significantly reduced and this phenomenon is thought to be related to the influx of immunocytes in the late phase response. Oral corticosteroids inhibit this increased reactivity as well as the late phase response and the cellular influx. Repeated exposure to antigen maintains a constant inflammatory process in the nasal mucosa, as seen in perennial rhinitis.

7.2.19 Signal transduction in allergic rhinitis

The precise mechanisms underlying allergen-induced release of
inflammatory mediators in the nasal mucosa are unclear, although a number of triggering mechanisms have been postulated. The first (Classical) mechanism involves allergen-induced activation of specific epithelial cell surface receptors (probably IgE), which trigger intracellular signal transduction cascades, leading to alterations in cytokine gene transcription. A second mechanism effects by direct allergen induced irritation of mucosal nerve endings, resulting in neurogenic inflammation. Inflammatory airway disease appears to have a common initiating pathway with recruitment of adhesion molecules to sites of inflammation (Montefort, Holgate et al. 1993). A third mechanism involves allergens with enzymatic properties (e.g. house dust mite allergen), producing direct damage to the epithelial cell membrane, thus stimulating the synthesis and release of inflammatory cytokines (Robinson et al., 1997). A similar mechanism has also been proposed for non-allergenic pollutant induced cytokine release, in which aero-pollutants produce epithelial cell damage, leading to free radical and oxidant formation.

7.2.20 **Irritative rhinitis**

An important and often neglected area of nasal pathology is irritative rhinitis. Irritative rhinitis is the development of typical rhinitis symptoms
in response to a variety of non-allergic stimuli or irritants. These include cigarette smoke, traffic fumes, perfumes, cold dry air, domestic cleaning agents and other aero-pollutants. Irritants in high concentration may provoke the acute nasal symptoms in normal subjects, however, in those with existing nasal symptoms, lower levels of exposure may provoke symptoms. This is referred to as non-specific nasal hyper-reactivity, and is correlated with an increase in the number of eosinophils and an increase in vascular permeability in the nasal mucosa of allergic rhinitics (Terr, 1991).

Fig.6  Role of IL-6 in general acute phase response

![Role of IL-6 in general acute phase response](www.nature.com, Jan 2008)
Fig. 7  World Allergy Organisation- prevalence of rhinitis

![Fig. 7 World Allergy Organisation- prevalence of rhinitis](https://www.worldallergy.org)

Courtesy of [www.worldallergy.org](http://www.worldallergy.org), Jan 2008

Fig. 8  Role of cytokines in rhinitis

![Fig. 8 Role of cytokines in rhinitis](https://www.nature.com)

Courtesy of [www.nature.com](http://www.nature.com), Jan 2008
Fig. 9  Cytokine reactivity and mast cells

Courtesy of www.nature.com, Jan 2008
Mechanism of Immediate Hypersensitivity Response

Courtesy of www.nature.com, Jan 2008
7.3 Allergic rhinitis

7.3.1 Allergic rhinitis- Clinical features

Rhinitis is an inflammatory disease of the nasal mucous membrane.

Although virtually unrecognized 200 years ago, rhinitis has become a common disease since the industrial revolution and particularly the 20th century (Vining, 1998). The reason for this dramatic increase is not clear however rhinitis is frequently mis-diagnosed and under-diagnosed. Increased awareness of Hay fever may well account for the sharp rise in consultations regarding this (Sibbald and Rink 1991). Epidemiological data indicates, however, environmental pollution may play a role.

7.3.2 Classification

Allergy is the most commonly identified cause of rhinitis. The nasal inflammatory process may arise from a number of systemic diseases and anatomical abnormalities as well as non-allergic stimuli.

A clinical classification of rhinitis has been updated by the ARIA Guidelines in 2008 as follows:

1. ‘Intermittent’- symptoms present for
   - <4 days a week
   - Or for <4 consecutive weeks

2. ‘Persistent’ means that the symptoms are present
• More than 4 days a week
• And for more than 4 consecutive weeks

3. ‘Mild’ that symptoms are present but not troublesome and that none of the following items are present:
   • Sleep disturbance
   • Impairment of daily activities, leisure and/or sport
   • Impairment of school or work

4. ‘Moderate/severe’ means that one or more of the following items are present:
   • Sleep disturbance
   • Impairment of daily activities, leisure and/or sport
   • Troublesome symptoms

7.3.3 Characteristics of seasonal allergic rhinitis
Seasonal allergic rhinitis (SAR), also known as Hay Fever, is defined as nasal inflammation characterised by itching of the respiratory tract mucous membrane, sneezing rhinorrhoea, post-nasal drip and nasal blockage with an identified seasonal trigger such as tree pollen, grass pollen or mould.
7.3.4 **Characteristics of perennial allergic rhinitis**

Perennial allergic rhinitis (PAR) is characterized by year-round nasal inflammation with a defined perennial trigger, commonly dust, feathers or animal dander. The symptoms are similar to SAR although there is a tendency towards increased nasal blockage and fewer symptoms of itching, as the nasal mucosa becomes increasingly thickened by chronic cellular influx oedema.

Other symptoms include anosmia, conjunctivitis and facial pain.

It is important to note that in the majority of case, aetiology is often not clear-cut and overlap may exist between precipitating factors. An example of this is a patient with PAR may suffer seasonal exacerbations during the grass pollen season (typically June to July) together with flare-ups attributable to smoke, perfume or traffic fumes (Djukanovic, Feather et al. 1996). Risk factors such as current urban residence or birthplace, over-crowding, nasal septal deviation, chronic rhinosinusitis and polyposis appear to result in an increased risk of PAR (Min, Jung et al. 1997).

7.3.5 **Physical findings in allergic rhinitis**

Physical abnormalities are not a major feature of the disease. Classically there is a pale, boggy and bluish nasal membrane.
7.3.6 **Investigations**

7.3.6.1 Skin prick testing

Proof that nasal allergy is present requires the demonstration of allergen-specific IgE in the patient. The most widely used technique is the skin prick test in which the skin is pricked through a drop of antigenic extract using a specifically designed needle, introducing approximately 5\(\mu\)l of extract into the skin. The Type I hypersensitivity wheal and flare reaction is compared with the results of negative (diluent) and positive (histamine) controls.

7.3.6.2 Scratch Testing

The test antigen is placed on the area of skin scratched to remove keratinized layers. This test lacks specificity and sensitivity and is now no longer recommended.

7.3.6.3 Intra-dermal Dilution Testing

Standard quantities of a variety of antigen dilutions are placed intra-dermally by injection. This enables identification of the exact starting dose but cannot predict the final optimum therapeutic dose. It is considered as good as, if not better than, skin-prick testing. It does entail increased time and personnel costs and thereby is not commonly available.
7.3.6.4 Blood tests

1) RAST
The discovery of IgE as the antibody for the classical immediate allergic reaction ((Ishizaka, Ishizaka et al. 1966) lead to the development of the in vitro Radioallergoabsorbent test (RAST) for its detection in serum (Wide, Bennich et al. 1967). In vitro tests are considered more specific but less sensitive than skin testing and in the UK are generally used only if there is a contradiction to skin prick testing e.g dermatographia or inability to stop antihistamines.

2) Blood eosinophil count
This test has been related to history since eosinophilia is often found in rhinitis but is not as striking as asthma, and a normal eosinophil count does not rule out a diagnosis of allergic rhinitis.

7.3.6.5 Dust and perennial allergic rhinitis
Dust is the major cause of perennial allergic rhinitis (PAR) in sensitive individuals. “House dust” as a unique antigen does not exist but rather contains antigens such as dust mite products, animal dander, mould and cockroach.

House dust mite
The most important allergens in UK “house dust” are the products of dust mites Dermatophagoides pteronyssinus and Dermatophagoides farinae.
The dust mite thrives in the modern household, since optimum environmental conditions for mite growth include temperature 17-25°C and relative humidity >50%. Food sources for mites includes scales from human skin or the fungi that grow on them, and mites are found predominantly in bedding, upholstery, furniture and carpets (Tovey, Chapman et al. 1981). Two major allergen groups (1 and 2) have been identified as most important, because the majority of mite-sensitive patients demonstrate immediate hypersensitivity to them. These major allergens appear to be digestive enzymes secreted in the dust mite faeces (Tovey, Chapman et al. 1981; Chua, Stewart et al. 1988; Thomas, Heap et al. 1991; Yasueda, Mita et al. 1993).

The allergens are soluble proteins with a molecular weight of 14-25kDA, which easily penetrate the nasal mucosa to react with specific IgE (Roche, Chinet et al. 1997).

The majority of patients with dust allergy experience symptoms once the number of dust particles in the air reaches 50/m³.

7.3.6.6 Epidemiology of allergic rhinitis

Burden & prevalence of disease

Although the effects of PAR and SAR are unlikely to be life threatening, one must bear in mind the great burden of disease and loss of quality of
life for the estimated 6 million UK sufferers (Frank and Rabin 1989).

In the USA direct and indirect costs of allergic rhinitis total well above $500 million per annum, with the disease estimated to be responsible for 10 million days lost from work and 8 million visits to the physician (NSAID Task Force Report, 1979).

The prevalence of diagnosed SAR amongst patients consulting general practitioners is reported to be 19.7 per 1000 in England and Wales (Ross and Fleming 1994)

Estimates of the prevalence of allergic rhinitis in different countries vary between 0.5-28% in children and 0.5-15% in adults (Aberg, Hesselmar et al. 1995; Strachan, Butland et al. 1996).

Within the UK a study of 17,414 children followed to age 23 years showed that the prevalence of self reported allergic rhinitis was 14.1% in Scotland rising to 20% in south-east England (Strachan 1989).

This north/south gradient was similarly demonstrated by Fleming & Crombie who showed that the proportion of the population consulting their General Practitioner for SAR was higher in the south than in the north of Britain (Fleming 1989).

There is little doubt that the prevalence of allergic rhinitis has increased dramatically over the last 30 years, as illustrated by the epidemiological studies of school children in Aberdeen, (Ninan and Russell 1992) and in
eastern Switzerland (Burkholter & Schiffer, 1995).

7.3.6.7 Associated conditions and complications

There is a well known association between allergic rhinitis, asthma and eczema (Sibbald and Rink 1991). The prevalence of asthma amongst persons with SAR are four to six fold higher than the general population (Broder, Higgins et al. 1974). The prevalence of allergic rhinitis amongst asthmatics is 28-50% as compared with its estimated population prevalence of 10-20% (Weeke 1987; Spector 1997). Allergic rhinitis may be complicated by a number of conditions including nasal polyposis, infectious rhinosinusitis and otitis media.

7.3.6.8 Treatment

Management of allergic rhinitis may appear complex. The importance of early treatment has been discussed by Rudack et al in 2007 (Rudack, 2007). Allergic Rhinitis is associated with other co-morbidities and early appropriate treatment is imperative. Anti-histamines and glucocorticoids remain a keystone in the medical management however more recently immunotherapy has been the sole treatment. There is a broad spectrum of pharmacotherapeutic groups that are currently available which will be discussed further now alongside non-pharmacotherapeutic treatment.
Environment changes

Environmental control is considered the first line in the management of allergic rhinitis. Reducing exposure to high dose pollen counts and minimising household pollen by closing windows and using air conditioning. Furnishing living areas without carpets or plants with a minimum of upholstery is advisable.

Pharmacological therapy

Antihistamines provide H1- receptor blockage. First- generation antihistamines have significant sedating effects and can produce tachyphylaxis. Second generation antihistamines do not produce this problem. Pseudo-ephedrine is effective for nasal obstruction. It can be used alone or in combination with anti-histamines. Topical steroid sprays are very effective for nasal allergy symptoms. Several new anti-histamine and mast cell stabilizers are also available. Anticholinergics are also used for chronic rhinorrhea. Oral antileukotrienes agents such as montelukast have also proved very helpful.

Allergy immunotherapy (desensitization) is indicated for individuals who fail environmental control and pharmacotherapy. Therapy is based upon antigen sensitivity delineated by allergy testing, either in-vitro or skin (Bradding et al, 1999). It is however a costly and inconvenient treatment requiring weekly injections for up to two to five years with a potential risk of anaphylaxis. Contraindications include poorly controlled asthma and induction during pregnancy. There is a 60% long-term control of symptoms.
The ARIA guidelines (Allergic Rhinitis and its Impact on Asthma) have provided a practical and staged approach to treatment. Intranasal steroids remain the single most effective therapeutic agent in the treatment of allergic rhinitis.

7.4 CHRONIC RHINOSINUSITIS

Chronic rhinosinusitis is one of the most prevalent chronic illnesses affecting the western world. These infections are the leading cause of acute morbidity and of school and work absenteeism.

It accounts for 20% of adult antibiotic prescriptions were written for a diagnosis of acute sinusitis. For the purposes of this thesis we focus on skin prick test positive allergic rhinitis and chronic rhinosinusitis confirmed by a pre-operative CT-scan, (Hadley 2001).

Classification of rhinosinusitis has been clarified by EPOS (European Paper on Rhinosinusitis and Nasal Polyps).

Rhinosinusitis is defined as inflammation of the nose and paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip):

+/- facial pain/pressure

+/- reduction or loss of smell

And either

• Endoscopic signs of
- polyps and/or
- mucopurulent discharge primarily from middle meatus and or
- oedema/mucosal obstruction primarily in middle meatus
And/or

- CT changes:
  - Mucosal changes within the ostiomeatal complex and/or sinuses

Severity of the disease can be divided into mild, moderate and severe based on total severity visual analogue scale (VAS) score (0-10cm):

Duration of disease:

Acute: <12 weeks with complete resolution of symptoms

Chronic: >12 weeks symptom without complete resolution of symptoms

The management costs involved in chronic rhinosinusitis are significant in terms of both medications and work time lost, and thereby research is needed to identify the patients for potential complications. Injudicious usage and indiscriminate over-prescriptions of antibiotics have fostered the rapid development of penicillin-resistant organisms over the past two decades. Drug resistant Streptococcus pneumoniae and beta-lactamase-producing Haemophilus influenza and Moraxella catarrhalis are perceived to be the main focus of attention, forcing the need to consider alternatives in antibacterial management. Appropriate medical management of this common problem requires a systematic approach with
consideration of adjunctive therapy.

Chronic rhinosinusitis is usually defined as sinus infection persisting for more than 3 months. Symptoms are perhaps more clearly defined than in allergic rhinitis. These include nasal stuffiness, postnasal drip, anosmia, facial fullness and malaise (Bajracharya et al, emed- Jan 2003). Allergic and non-allergic rhinitis, anatomic obstruction of the ostiomeatal complex are known risk factors (Steinke and Borish 2004).

Knowledge of the anatomy of paranasal sinuses is an essential tool in understanding the pathophysiology and management. Four pairs of sinuses are lined with ciliated pseudo-stratified columnar epithelium. Goblet cells are columnar cells. The mucous is attached directly to the bone thereby involvement of bone, orbital and intracranial compartments occur in inadequately treated cases. The maxillary, frontal and anterior ethmoid sinuses drain through their ostia located at the ostiomeatal complex within the middle meatus.
Fig. 11  Anatomy of the paranasal sinuses

Courtesy of www.merck.com, Jan 2008

Fig. 12  Nasal and paranasal sinuses
The posterior ethmoid and sphenoid sinuses open into the superior meatus respectively. The maxillary ostium connects to the nasal cavity by mucociliary clearance. The inferior floor of the maxillary sinus is the tooth-bearing part of the maxilla and dental infections can easily extend to the maxillary sinus. Even though usually colonized by bacteria the sinuses are typically sterile. Mechanical obstruction at the ostiomeatal complex secondary to anatomic factors or mucosal oedema arising secondarily to a acute viral or allergic rhinitis often triggers the stasis of secretions within the sinuses. This mucous stagnation effects the growth of various pathogens. Initially, resulting acute sinusitis involves only one type of aerobic bacteria however a mixed flora and anaerobic organisms and occasionally fungus contribute to the pathogenesis. Chronic sinusitis ensues in those where acute sinusitis has not responded to treatment or those who have not received treatment.

The role of bacteria in the pathogenesis of chronic rhinosinusitis is currently in question. In the United States chronic rhinosinusitis affects approximately 32 million persons each year and accounts for 11.6% sickness in offices. It ranks fifth compared to all diseases in frequency of antibiotic use. Internationally, chronic rhinosinusitis is a common disease worldwide, particularly in places where the atmosphere is damp.
Temperate climates along with higher concentrations of pollens are associated with a higher prevalence as in the northern hemisphere. Because of its persistent nature, chronic sinusitis can become a significant cause of malaise and reduced quality of life and productivity of the affected person. Chronic sinusitis is associated with complications such as brain abscess and meningitis which can produce significant morbidity and mortality.

7.4.1 **Aetiology of chronic rhinosinusitis**

Chronic rhinosinusitis is observed in all races and both sexes are affected equally. All age groups are affected.

7.4.2 **Clinical history of chronic rhinosinusitis**

Chronic rhinosinusitis manifests more subtly than acute sinusitis. The typical symptoms of acute sinusitis, namely fever and facial pain, are absent. Patients usually present with the following symptoms:

- Nasal stuffiness
- Nasal discharge
- Postnasal drip
- Facial pain
- Facial fullness, discomfort and headache
- Chronic unproductive cough
- Hyposmia
- Sore throat
- Fetis breath
- Malaise
- Exacerbation of asthma
- Dental pain
- Visual disturbances
- Sneezing
- Stuffy ears
- Unpleasant taste
- Fever of unknown origin

7.4.3 Classification and Diagnosis of chronic rhinosinusitis

The European Position on Rhinosinusitis and Nasal Polyps in 2007 is discussed above. Severity of the disease can be divided into mild, moderate and severe based on visual analogue score (VAS) (0-10cm)

Mild= VAS 0-3
Moderate=VAS>3-7
Severe= VAS>7-10

To evaluate the total severity the patient is asked to indicate on VAS the answer to the question:
HOW TROUBLESOME ARE YOUR SYMPTOMS OF RHINOSINUSITIS?

10cm

Not troublesome  Worst thinkable troublesome

AVAS > 5 is considered to affect quality of life

Duration of the disease

Acute: < 12 weeks with complete resolution of symptoms

Chronic > 12 weeks symptoms without complete resolution of symptoms - may also be subject to exacerbations

Diagnosis

Symptoms for less than 12 weeks.

Sudden onset of two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip):

+/- facial pain/pressure

+/- reduction /loss of sense of smell

With free intervals if problem is recurrent; validation by telephone or interview asking questions on allergic symptoms, i.e, sneezing, watery rhinorrhoea, nasal itching and itchy watery eyes

Chronic rhinosinusitis has also recently been classified into four types based on its pathophysiologic process, (Steinke and Borish 2004).

A minority of patients have chronic infectious sinusitis. A second form
chronic inflammatory sinusitis is believed to arise from chronic sinus ostia occlusion with resulting recurrent infections and damage to the sinus epithelium. Chronic inflammatory infiltration ensues with mononuclear cell proliferation.

Allergic fungal sinusitis is epithelium a severe T-helper 2 (Th2) inflammatory process in response to usually benign fungal colonisation and provides a third category. Chronic hyperplastic eosinophilic sinusitis (CHES), is an inflammatory disease hallmarked by a prominent accumulation of eosinophils, is considered a fourth subtype. CHES is frequently associated with nasal polyposis, allergic sensitization, asthma, and aspirin sensitivity. Cysteinyl leukotrienes are able to induce vascular leakage, mucous secretions, myofibroblast proliferation, and eosinophil recruitment, adhesion and survival, (Steinke and Borish 2004). These are all important in the pathophysiology of CHES.

An open-labelled study has shown improvement in nasal symptom scores but not in objective parameters in 32 patients with CRS treated with montekulast, a leukotriene-modifying drug. This drug type appears to be safe and attractive option for the treatment of CHES. Leukotriene treatment of this last category, CHES, is the focus of many reviews. Surgery is often beneficial for anatomic defects or chronic inflammatory sinusitis, however have been disappointing for CHES, (Kennedy 1992; Lavigne, Nguyen et al. 2000). Important advancements include the anti-inflammatory
properties of macrolide in CHES. a review article has been published by (Scadding 2004).

The underlying cause of CHES is unknown and its chronic inflammatory disease characteristic by not only eosinophils but also fibroblasts, mast cells, goblet cells, and Th2 lymphocytes. It does not appear to be an infectious process, but bacteria and fungi may act as immune stimulators or super-antigens.

There does appear to be a relation between CHES and allergic disease. Steinke and Borish outlined their work on the systemic inflammation of allergic disease and how it interacts with sinusitis in a recent review. Twenty-five to 58% of individuals with sinusitis have allergic rhinitis and allergic sensitivity, especially to perennial allergens, which increase the risk for CHES (Savolainen 1989; Emanuel and Shah 2000; Gutman, Torres et al. 2004). Half of CHES patients also have clinically evident asthma. It has been argued that CHES and asthma share similar histopathological features and are the same inflammatory process manifested in different sites, (Ponikau, Sherris et al. 2003). In recent years the focus has been on the association between gastrointestinal reflux disease (GERD) and its role in upper and lower airway disease. There are several studies supporting a worsening role for GERD in asthma but there is fewer data on sinusitis. A review of the literature performed by (Weaver 2003) found a modest evidence for a positive association between GERD
and sinusitis. A small open-label pilot study was performed to examine the effects of omeprazole in CRS patients, using GERD patients as controls. There was a high prevalence of GERD in CRS patients and treatment with proton pump inhibitor had an improvement in sinus symptoms, (DiBaise, Olusola et al. 2002). This area requires further investigation.

Clarithromycin has been shown to inhibit transforming growth factor and nuclear factor from nasal biopsy samples as well as the effects on suppressing interleukin-5, interleukin-8, and granulocyte-macrophage colony-stimulating factor was equal to prednisolone in nasal biopsy samples of patients with CRS (Wallwork, Coman et al. 2004). There has been limited in vivo work in the United States however a good deal of data is being produced in Japan. Fifty-six patients had an overall improvement based on subjective and objective criteria, (Katsuta, Osafune et al. 2002). Tamaoki (Tamaoki 2004) have shown that LPS up-regulates the expression of ICAM-1 when added to cultured rat tracheal epithelial cells. LPS endotoxin comprises a major portion of cell walls of gram-negative bacteria and interacts with a variety of cell types, including neutrophils, basophils and monocytes. Studies have demonstrated that LPS increases microvascular permeability, neutrophil chemotaxis, accumulation into airway wall and, hence neutrophil airway inflammation.

7.4.4 Physical signs of chronic rhinosinusitis

A diagnosis chronic rhinosinusitis is made if symptoms last longer than 12 weeks
Two or more symptoms, as described above, are essential. One of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip):

+/- facial pain/pressure

+/- reduction or loss of smell

With validation by telephone or interviews asking questions on allergic symptoms; i.e. sneezing, watery rhinorrhea, nasal itching and itchy watery eyes. If positive, allergy testing should be performed.

Physical examination may reveal a variety of findings.

- Pain or tenderness on palpation over frontal or maxillary sinuses.

- Oropharyngeal erythema, purulent secretions

- Dental caries

Endoscopic examination findings:

- Nasal mucosal erythema, oedema

- Purulent secretions

- Nasal obstruction due to deviated nasal septum or hypertrophied turbinates

- Nasal polyps

Ophthalmic manifestations include:

- Conjunctival congestion

- Lacrimation
- Proptosis, extraocular muscle palsies and visual disturbances (when orbital extension).

### 7.4.5 Causes of chronic rhinosinusitis

The bacterial pathogens and their roles are well defined in the aetiology of acute sinusitis. Streptococcus influenzae and Mozarella catarrhalis account for more than 70% of cases of acute sinusitis. The role of viruses in the aetiology of acute sinusitis has been documented. Bacterial sinusitis complicated up to 20% of viral rhinitis cases. While the microbiology of acute sinusitis has also been established, researchers disagree on the microbiology of chronic rhinosinusitis. Some studies have documented anaerobes as the prominent pathogens in chronic rhinosinusitis, while others contradict this.

The reasons for the variable growth of microbes in the samples obtained from chronic sinusitis reflect exposure of patients to various broad-spectrum antibiotics as well as to the difference in the exact role of these microbes in the pathogenesis of chronic sinusitis.

Increasing attention is being focused on the ostio-meatal obstruction, allergic, polyps, occult and dental diseases, while the role of bacteria is being reduced to that of opportunistic colonizer.

The following bacteria have been reported in samples obtained through endoscopy or sinus puncture sinusitis:
1 Staphylococcus aureus
2 Coagulase-negative staphylococci
3 H influenzae
4 M catarrhalis
5 S pneumoniae
6 Streptococcus viridans
7 Streptococcus intermedius
8 Pseudomonas aeruginosa
9 Nocardia species
10 Anaerobic bacteria

The following fungi have been reported in samples obtained through endoscopy or sinus puncture
1 Aspergillus species
2 Cryptococcus neoformans
3 Candida species
4 Sporothrix schenckii
5 Alternaria species

The following conditions and risk factors predispose patients to the development of chronic rhinosinusitis:
1. Anatomic abnormalities affecting the ostiomeatal complex (e.g., septal deviation, paradoxical turbinates, concha bullosa, Haller cells)

2. Allergic rhinitis

3. Nasal polyps

4. Nonallergic rhinitis (e.g., vasomotor rhinitis, rhinitis medicamentosa, cocaine abuse)

5. Nasotracheal intubation

6. Nasogastric intubation

7. Hormonal (e.g., puberty, pregnancy, oral contraception)

8. Tumoral obstruction

9. Immunologic disorders (e.g., common variable immunodeficiency, immunoglobulin A deficiency, AIDS)

1. Cystic fibrosis

2. Primary ciliary dyskinesia, Kartagener syndrome

3. Wegener’s granulomatosis

4. Repeated upper respiratory tract infections

5. Smoking

6. Environmental pollution

7. Gastroesophageal reflux disease

8. Perichondritis/significant dental disease
It is important to consider other co-existing conditions that are associated with chronic rhinosinusitis.

These include the following:

1. Temporomandibular Joint Syndrome
2. Asthma
3. Other chronic rhinitis
4. Nasal and sinus cavity tumours
5. Facial pain attributable to other causes
6. Nasal polyps

It has been shown by many studies that there is no correlation between nasal flora and culture from sinuses (Lacroix, Ricchetti et al. 2002). Nasal swab cultures have no diagnostic value. Occasional, an abundance of eosinophils in the nasal smear may point to the allergic nature of the mucosa. Specimens obtained from sinus openings through an endoscope correlate well with specimens obtained from respiratory brushings. Routine blood cell counts and sedimentation rates are generally unhelpful. In severe cases, blood cultures, including fungal blood cultures, may be helpful. Allergy testing must be carried out if a sensitivity is thought to be the underlying cause.
7.4.6 Investigation of chronic rhinosinusitis

The cornerstone for diagnostic workup of chronic sinusitis is the radiological examination.

Plain radiograph
The routine radiograph has limited value in the evaluation of sinusitis however it has been thought that mucosal thickenings or sinus opacities may be observed. Air fluid levels are not a common feature in chronic sinusitis and plain films do not show ethmoid sinuses or the ostiomeatal complex well.

7.4.6.1 CT Scan
Contrast-enhanced CT scan is the current radiologic standard for the evaluation of sinus. Scanning may be prohibitively expensive or medically unnecessary. CT scans are usually indicated after failure of maximal medical therapy and before surgical planning and in exclusion of possible neoplasms. A coronal CT scan of the sinus correlates best with the surgical approach, permitting visualization of the ostiomeatal complex, sinus cavities and surrounding structures such as the orbit, cribiform plate, and dental pathologies are visualized well. Specific entities in the sinus cavity such as polyps can be identified.

A CT scan combined with endoscopic examination helps the surgeon to make operative decisions. Most centres now offer limited sinus CT scans.
consisting of 5-12 coronal cuts.

7.4.6.2 MRI

MRI is generally reserved only for complex cases. Soft tissue contrast is better with MRI. Neoplasms, orbital and intracranial complications as well fungal sinusitis can be better evaluated.

Procedures

Appropriate cultures of the ostiomeatal complex region should guide otolaryngologists to a proper choice of an antimicrobial regimen, but culture of nasal secretions traditionally has not been correlated with the findings on antral puncture. The gold standard of diagnosis has traditionally been culture of the maxillary sinus via antral puncture, however, recent evidence has linked the association of endoscopic-guided cultures (obtained with fine- wire swabs) to those of antral aspirates. 85.75% of endoscopic-guided cultures corresponded to bacteria present in the maxillary antrum. More recently Vogen et al noted that endoscopically guided meatal cultures were accurate and identified the predominant bacterial pathogen in 90% of cultures. Laboratory evaluation of rhinosinusitis may include nasal cytology (looking for neutrophils), sweat chloride tests and mucociliary transport studies, which evaluate the timing of the passage of saccharin through the nose to the posterior oropharynx.
7.4.7 Management

Treatment should be based on the severity of symptoms

*Mild* - VAS 0-3 *Moderate to Severe* - VAS>3-10

Treat with topical steroids, nasal douching/lavage.

In failure to improve then supplement a three month course of macrolides. If the symptoms are refractory to medical treatment, a CT scan is required to identify indications for surgery.

Ragab et al conducted the first prospective randomized trial evaluating and comparing surgical and medical management of polypoid and non polypoid chronic rhinosinusitis (Ragab, S. M., V. J. Lund, et al. (2004). "Evaluation of the medical and surgical treatment of chronic rhinosinusitis: a prospective, randomised, controlled trial." Laryngoscope 114(5): 923-30). Their work analysed the outcome of ninety patients over a one year period with CRS using visual analogue scores(VAS), the Sinonasal Outcome Test-20(SNOT-20) and other methods of post treatment assessments. The study concluded that medical treatment should be the mainstay of management in the form of topical steroid, nasal douche with a three month course of macrolide antibiotic. In refractory cases surgical treatment may be applied. Ragab et al also concluded the presence of polyps was not in fact a poor prognostic factor in the efficacy of either treatment.
7.4.8 Medical Management

Medical therapy is in some cases considered an adjunct to surgical treatment and is directed toward colonizing infections, reducing oedema of sinus tissues and facilitating the drainage of sinus secretions. The role of bacteria is debatable, however, when diagnosed early and intensively treated with oral antibiotics, topical nasal steroids, a number of patients have relief from symptoms and many can be cured. It is imperative to assist medical therapy, in particular if unsuccessful, by evaluating the following:

1. Control of predisposing factors such as risk factors and aetiologies for the development of chronic sinusitis or modify these factors in the management of chronic sinusitis.

2. Viral upper respiratory tract infections; Reduce viral exposures by improved personal hygiene. Respiratory tract infections are controversial.

3. Environmental factors: Reduce exposure to dust, moulds, cigarette smoke and other environmental hazards.

4. Allergic rhinitis: Environmental control, anti histamines, cromolyn, topical steroids, or immunotherapy.

5. Patients with adult chronic sinusitis may benefit from control of GERD. GERD has increasingly ailments such as asthma and chronic sinusitis. The exact relationships and mechanisms are currently
under evaluation.

6 Immunodeficiency states: Appropriate control of various congenital and acquired immunodeficiency.

7.4.9 Symptomatic Measures

Symptoms may be relieved with topical decongestants, topical steroid, antibiotics and nasal saline douches. Steam inhalation and nasal saline irrigation may help by moistening dry secretions, reducing mucous.

Fungal sinusitis

Fungal sinusitis can manifest in different ways. Acute invasive fungal sinusitis is observed in patients who are immune-suppressed or diabetic. Aspergillus, Mucor and Rhizopus are the main cause. The condition requires urgent work up and aggressive medical management. Chronic fungal sinusitis is usually observed in patients who are immune-competent. Surgical drainage may necessitate. Mycetomas or fungal balls may be asymptomatic or may manifest as chronic sinusitis. Allergic fungal sinusitis usually manifests as nasal polyps in allergic sinusitis. Surgical treatment may again be necessary. Persistent or recurrent episodes of sinusitis despite appropriate medical therapy necessitate referral to a specialist for endoscopic evaluation and CT scanning, mandatory to exclude surgically amenable conditions. In the event of orbital involvement, an ophthalmological opinion is necessary.
7.4.10 Pharmacotherapy

7.15.1 Topical decongestants

These are alpha-adrenergic agonists that act by constricting dilated mucosal capillaries. Pseudoephedrine hydrochloride, phenylephrine hydrochloride, and xylometazoline are available for use in the topical form. The resulting vasoconstriction of mucosal capillaries promotes shrinkage of the swollen nasal mucosa. All adrenergic topical preparations should be with caution as topical agents can produce rebound vasodilatation on discontinuation and rhinitis medicamentosa with prolonged use.

7.4.10 Topical corticosteroids

These are particularly effective for chronic sinusitis associated with an infection. Recent studies show that the medical management of bacterial rhinosinusitis can be enhanced with the addition of intranasal steroids. Topical intranasal steroids have marked anti-inflammatory actions to reduce the vascular permeability and to inhibit the release of chemical mediators, especially histamine, leukotienes and others (Mullol, Lopez et al. 1997). They reduce cellular influx to the inflammatory site and modify both the early and late-phase responses in rhinitis. The same principles hold true in sinusitis; topical intranasal steroids should have marked beneficial effects due to similarity of respiratory epithelium in the nose and the paranasal
sinuses. Topical nasal steroids are advocated in hyperplastic polypoid rhinosinusitis (Damm, Jungehulsing et al. 1999). Hamilos, Thawley et al. 1999, demonstrated gradual response of the nasal polyps to intranasal steroids and showed that they also reduced nasal polyp inflammation but not the expression of pro-inflammatory cytokines.

Damm et al. 1999 combined the use of oral steroids and intranasal steroids and found markedly reduced polypoid disease, but this medication did not affect the anterior ethmoid area and did change the need for consideration of surgery.

Topical steroids along with systemic antibiotics are now key components of the medical armamentarium. Fluticasone propionate is an example (Hamilos, Thawley et al. 1999) and is uses in particular with allergic and vasomotor rhinosinusitis and also rhinosinusitis medicamentosa as well as in the prophylaxis of nasal polyps. Plasma concentrations remain very low following long-term application.

7.4.12 Antihistamines

Allergic rhinitis is a cause for rhinosinusitis. Antihistamines may be beneficial in adjunctive therapy for the patient with a history of allergic rhinitis. The type of antihistamine is controversial. The first generation of antihistamines may tend to reduce the rhinorrhea associated with the inflammation, but drying effects of these products thicken sinus secretions and possibly slow mucociliary clearance. The newer second-
generation antihistamines (acrivistine, cetirizine, fexofenadine, and loratidine) would be favoured in patients with a history of allergy.

Topical antihistamines currently available (azelastine, levocabastine) are extremely potent histamine blockers, but no controlled studies of their utilization in rhinosinusitis are available at this time.

7.4.13 Topical nasal sprays

Nasal saline spray and steam inhalation help by moistening dry secretions and their viscosity. Substantial symptomatic relief is gained in some patients. Saline spray loosens mucous secretions to help extrusion of mucus from the nose and sinuses.

7.4.14 Mast cell stabilizers

Cromolyn sodium inhibits degranulation of sensitized mast cells following their exposure to specific antigens.

7.4.15 Expectorants

Although there are no controlled studies on the efficacy of mucolytics in chronic sinusitics, they have been helpful in ameliorating some symptoms.

Inpatient care is indicated if orbital or intracranial complications ensue.

Patients who are immunosuppressed and paediatric patients may need
in-patient care depending on the progression of infections.

Continued outpatient medical treatment with nasal decongestants and topical steroids is important in monitoring the progress of cases.

Certain conditions cause a predisposition to chronic sinusitis.

7.4.16 Antibiotics

The goals of pharmacotherapy are to eradicate the infection, reduce morbidity and prevent complications. Antibiotics have traditionally been used as effective eradicators of bacterial sinus infections. It has been shown that antibiotics improved symptoms and decreased or eradicated bacteria from the maxillary sinus (Gwaltney, Wiesinger et al. 2004). When used appropriately, antibiotics are effective in the management of bacterial respiratory tract infections, leading to more rapid resolution of infection and relief of symptoms. They can also help prevent the progression of disease from acute to chronic manifestations.

Antibiotic regimens are based on empiric knowledge of the common pathogens responsible for the disorder, predominantly Streptococcus pneumoniae, Haemophilus influenza, and Moraxella catarrhalis, and penicillin, amoxycillin, and ampicillin have been standard therapies in non-allergic patients. Other antimicrobials, including the cephalosporins, macrolides, sulph-based antibiotics, and the fluoroquinolones, have been utilized in comparative studies over the years. Studies have documented
the prevalence of intermediate and resistant S.pneumoniae to penicillin over the past two decades. The resistance patterns have been a major concern for health care providers who used to depend on the susceptibility of the most common pathogens to penicillins. Bacteria develop resistance to penicillin by alteration of penicillin-binding proteins or by production of beta-lactamase production has increased and remains at approximately 405 for H.influenza and at 98% for M.catarrhalis. Brook 2001; Brook and Frazier 2001; Brook 2004 note that beta-lactamase-producing bacteria can express their pathogenicity directly through their ability to cause infections and indirectly by production of enzymes. This protects penicillin-susceptible pathogens from penicillin failure in the management of group-A-beta haemolytic streptococcal tonsillitis.

Overuse of antibiotics contribute to the resistance of microorganisms. Factors contributing to this include patient expectation, clinician time constraints, defensive medicine and avoiding the potential sequelae of not prescribing for a patient who has a bacterial infection. Studies in Finland showed that the consumption of erythromycin was related to an increase in the development of erythromycin-resistant group A streptococci. Yet, after 2 years a significant decline in macrolide-resistant group A streptococcus infection occurred after use of the macrolide antibiotics
was reduced. Klossek et al evaluated the types of bacteria implicated in chronic rhinosinusitis. Endoscopic-guided cultures were performed. They found approximately 25% of isolates produced beta-lactamase, resulting in a decrease in sensitivity to penicillin in 13% of isolates. Their findings showed that amoxycillin-clavulanate was the most active oral antibiotic in vitro.

Over the past few years, developments in the evaluation of pharmacokinetics (absorption, distribution, metabolism and excretion) of antimicrobials has been established. Pharmacodynamics relates to the concentration of the drug and its pharmacologic effect. Antimicrobials may exhibit either time-dependent killing or concentration-dependent killing. Time-dependent antimicrobials include the beta-lactams, macrolides, and clindamycin. Once these antibiotics reach a critical concentration, increasing the concentration further will not increase the rate or extend the bacterial killing. Concentration-dependent antibiotics need to achieve the a relative value well above the minimal inhibitory concentration of the bacteria. It is necessary for these antimicrobials to obtain a very high peak of concentration and they are not time dependent. The most recent data are from The Anti-microbial Treatment Guidelines for acute bacterial rhinosinusitis is established by the Sinus and Health Partnership. Based on the efficacy rates, the partnership developed
recommendations for antimicrobial therapy. These recommendations were appropriate for patients with mild disease who had received antibiotics in the previous 4-6 weeks and patients with moderate disease assuming severe disease would require intravenous treatment. The recommended initial antibiotic therapy for adult patients or children with mild bacterial rhinosinusitis is amoxycillin-clavulanate, amoxycillin, cefodoxime, or cefuroxime, based on efficacy data. Treatment regimens suggest 10-14 days for adequate duration therapy in patients who develop acute rhinosinusitis. Some have recommended an additional 7 days once symptoms improve. The duration of therapy remains unspecified as resistant bacteria can remain viable in a closed sinus long after effective antibiotic regimen has been in place. An initial 2-4 week trial of antibiotics may be reasonable after surgical management for uncomplicated chronic infection. Invasion of bone or deep structures may require a prolonged antibiotic course. Amoxicillin interferes with synthesis of muco-peptides during active multiplications, resulting in bactericidal activity in susceptible bacteria. Clarithromycin inhibits bacterial growth, possibly by blocking peptidyl t-RNA from ribosomes, causing RNA-dependent protein synthesis.
7.4.17 Macrolides

Studies of macrolides in chronic airways disease, such as diffuse pan-bronchiolitis (DPB) has led to improvements in pulmonary function (Banerjee, Khair et al. 2005). Similar benefits have been documented in Japanese studies of bronchiectasis, chronic bronchitis and sino-bronchial syndrome. Clinical and pathologic similarities between DPB and cystic fibrosis (CF) led to the investigation of macrolides for the treatment of CF. In vitro and in vivo studies of macrolides suggest that they inhibit the pulmonary influx of neutrophils and release of pro-inflammatory cytokines. They also protect the epithelium from bio-active phospholipids, and improve the transportability of airway secretions (Kikuchi, Hagiwara et al. 2002; Kikuchi, Hagiwara et al. 2003). The immune-modulatory effects of the macrolides also may be beneficial for the treatment of other chronic inflammatory conditions.

Interest in the immune-modulatory effects of macrolide antibiotics began with the observation that patients with severe asthma required lower doses of steroids if they also received troleandomycin (TAO).

As a primary anti-inflammatory activity, the macrolides appear to target nuclear transcriptional regulation. The stimulation of cells with various cytokines (e.g IL-8 and TNF-α) induces and activates a number of nuclear-binding proteins, which in turn trigger the transcriptional process to initiate and amplify the inflammatory response. Nuclear factor-kappa B is a protein that is essential for the transcription
of genes that encode a number of pro-inflammatory molecules that participate in the acute inflammatory responses, including TNF-α, ICAM-1, inducible nitric oxide synthase (Inos), IL-6, and IL-8. NF-kappa B is composed of two proteins, p50 and p-65.

The findings of studies by Ichiyama and colleagues concluded that macrolides modulate inflammatory activity in pulmonary epithelial cells and peripheral blood monocytes via a fundamental event or process. This quite possibly occurs at the level of gene transcription for pro-inflammatory cytokines.

Subsequently macrolides have been studied for other airway diseases including diffuse pan-bronchiolitis (DPB) and cystic fibrosis (CF) (Martinez and Simon 2004). Macrolides increase mucociliary clearance, improve sinusitis symptoms and decrease nasal secretions and polyp size in patients with sinusitis. They have also been shown to modify the inflammatory response associated with chronic sinusitis (Morikawa, Oseko et al. 1994). In patients with asthma, macrolides have been reported to reduce airway hyper-responsiveness and improve pulmonary function. They have been reported to reduce airway hyper-responsiveness as well as improving pulmonary function, and have historically been selected for their “steroid-sparing” effect. Data form patients with COPD have shown improvements in symptom scores and FEV_{1} after macrolide treatment. Macrolides, thereby, have the potential to
improve the outcomes of patients with inflammatory airways diseases. Large scale, placebo-controlled clinical trial in upper-respiratory inflammatory are warranted. Independent of their potent antimicrobial activity, 14-membered and 15-membered macrolides possess anti-inflammatory properties that may contribute to clinical benefits observed in patients with airway inflammation (Gotfried, M. H. (2004). "Macrolides for the treatment of chronic sinusitis, asthma, and COPD." Chest 125(2 Suppl): 52S-60S; quiz 60S-61S).

A number of studies have shown an improvement in the clinical symptoms of corticosteroid-dependent patients with asthma and a reduction in the corticosteroid dosage with concomitant TAO therapy. Pharmacokinetic studies indicate this may be due, in part, to inhibition of steroid metabolism. TAO therapy was shown to significantly prolong the serum half-life of methylprednisolone. In some reported studies some steroid-dependent patients were able to completely discontinue concomitant oral steroid therapy without worsening asthma severity, suggesting that TAO has directly anti-inflammatory activities.

Macrolide antibiotics also appear to be muco-regulatory, that is, they are able to decrease mucus hypersecretion in patients with airway disease without suppressing baseline physiologic secretions. Clarithromycin not only improves the solid composition of mucus but also reduces the
volume of nasal mucus secretion. Macrolides also affect neutrophil migration, the oxidative burst in phagocytes, the production of pro-inflammatory cytokines and eosinophilic inflammation.

Macrolides and clinical experience

Report of macrolide use in patients with chronic sinusitis come primarily from small, open-label case series. Consistent across the studies have been improvements in sinusitis symptoms, shrinkage in the size of nasal polyps, and a decrease in levels of pro-inflammatory cytokines in nasal secretions. Most of the clinical experiences demonstrating the favorable anti-inflammatory effects of macrolides on patients with chronic sinusitis have been published in the Japanese literature.

Data from the beginning of the last decade by Kikuchi et al in Japan documented improvement of sinusitis signs and symptoms in 50 to 100% of patients who are given macrolide therapy. The investigators treated 26 post-operative patients with persistent sinus symptoms following sinus surgery using erythromycin, 600mg per day for an average of 7.9 months. Also published in Japanese literature were the results of studies by Nishi et al and Suzuki et al. Following treatment with clarithromycin, 400mg daily for 4 weeks, significant improvements in mucociliary clearance, volume of secretions, cough frequency and dyspnoea-on-exertion were documented in 32 patients with sino-bronchial syndrome. Low-dose
roxithromycin (Rulid; Albert-Roussel Pharma GmbH; Wiesbaden, Germany) was shown to significantly improve the aeration of all four sinuses and to significantly reduce neutrophil and IL-8 levels in the nasal discharge of 12 patients with chronic sinusitis.

The first article describing the use of macrolide in patients with chronic sinusitis appeared in the English literature in 1996. Hashiba and Baba treated 45 chronic sinusitis patients with clarithromycin, 400mg daily for 8 to 12 weeks. Improvements in symptoms and rhinoscopic findings were directly related to the duration of macrolide therapy. The investigators improved rates of therapy. Clinical benefit in patients with chronic sinusitis also was observed following long-term administration of roxithromycin. Patients with chronic sinusitis often develop nasal polyps, which are either neutrophil-dominant (i.e containing abundant pro-inflammatory cytokines such as IL-8) or eosinophil-dominant. The 14-member macrolides (e.g erythromycin, clarithromycin), inhibited IL-8 secretion from cultures of human nasal epithelial cells harvested from the nasal polyps of patients with chronic sinusitis.

Yamada et al evaluated the effect of macrolide therapy on size of nasal polyps and IL-8 levels in the nasal lavage fluid of 20 patients (age range, 24 to 84 years; mean age, 57 years) with chronic paranasal rhinosinusitis. All patients had experienced >1 year of symptoms. During macrolide treatment
patients had a significant decrease in IL-8 levels, a critical cytokine in the pathogenesis of chronic rhinosinusitis. Clinical response correlated significantly with decreased IL-8 levels. In the group of patients in whom polyps were reduced in size during macrolide therapy, IL-8 levels were significantly higher at baseline and decreased by more than five-fold. In contrast, there was no difference in IL-8 levels before and after macrolide therapy in patients in whom polyps did not change in size. The investigators theorized that the shrinkage of nasal polyps by macrolide is related to the suppression of cytokines production by inflammatory cells in the paranasal sinus epithelium.

Low dose long term macrolide therapy may be one of the most exciting alternative treatments for CHES (Hashiba and Baba 1996). There are however no large-scale placebo-controlled clinical trials to confirm the results of several open-label trials. It must be noted that a recent study demonstrated the use of erythromycin by itself, and even further in combination with other drugs, significantly increases the risk of cardiac death, thereby raising concern about the recommendation for the routine use of this drug class.

7.4.18 Novel Therapy

Immunosuppressive therapy

There are several new immune-modifying drugs that have yet to be formally studied in CHES.
Immune modulation

Whilst traditional immunotherapy is clearly efficacious for allergic rhinitis, there have not been well-performed studies on CHES. Clinical trials are currently under way investigating methods of making immunotherapy safer and more effective.

Immunomodulation holds promise for the future of CHES, but in light of their cost and possible side effects they need to be better studied before being implemented in the clinical setting.

Environmental factors or allergic factors in these patients may be amenable to the following preventative measures:

- Reduce exposure to dust, moulds, cigarette smoke and other environmental chemical irritants.
- Environmental control, antihistamines, cromolyn, topical steroids or immunotherapy may be helpful in the medical management.

7.4.19 Complications

1 Orbital cellulitis

2 Cavernous sinus thrombosis

3 Intracranial extension (e.g, brain abscess, meningitis)

4 Mucocele formation

7.4.20 Prognosis

Satisfactory outcomes result when a patient with chronic sinusitis is
treated with aggressive medication. Functional endoscopic sinus surgery restores sinus health with complete or moderate relief of symptoms in 80-90% of patients.

It must be noted that nasal swab cultures do not correlate with sinus culture results. Always consider serious underlying conditions such as neoplasms and immunodeficiency states. Fungal sinusitis can be devastating in immunosuppressed patients.
8. HYPOTHESIS AND OBJECTIVES
8.1 **Hypothesis**

Rhinitis is an inflammatory condition affects the nasal mucosa.

The complex pathophysiology results in an often poorly understood condition. An overlap in symptoms of allergic rhinitis and chronic rhinosinusitis renders early diagnosis difficult. Management often is poor and consequently patients are often left feeling abandoned by their clinician.

The aims of this study are to further our understanding of the potential differences in the pathophysiological mechanisms of the two conditions and their interactions with macrolides whose function is complex.

1. My first hypothesis is that there are fundamental differences in baseline immunomodulatory activity between chronic rhinosinusitis and allergic rhinitis. The study aims to identify activity of specific cytokines, IL-6 and IL-8, both of which are already known to contribute to pathways of intra-cellular activity in each condition.

2. My second hypothesis is that macrolides influence activity of IL-6 and IL-8 in chronic rhinosinusitis and allergic rhinosinusitis and that there are differences in cytokine responses within each condition. ‘Macrolide – generated’immunomodulatory responses can have significant implications in the future treatment of these conditions.

The effects of macrolides on each group may help to formulate an improved understanding of these mechanisms and thereby determine
whether application in their treatment may benefit one or more specific group of patients.

8.2 Objectives

The objectives of this study are:

1. To culture HNECs as primary cultures from nasal turbinate biopsies.

2. To compare the constitutive release of two inflammatory markers (IL-6, IL-8) from well characterized non-atopic non-rhinitic subjects (Normal subjects); subjects with active perennial allergic rhinitis (Rhinitics); and non-atopic chronic rhinosinusitics.

3. To compare the quantity of IL-6, IL-8 released by HNECs obtained from well characterized Normal subjects, Rhinitics, Chronic Rhinosinusitics following exposure to Bacterial endotoxin (LPS), macrolides and both LPS and macrolides for a period of 24hours.
9.0 METHODS
9.1 METHODS

Studies have shown that airway epithelial cells and cell lines are capable of expressing and synthesizing a large variety of cytokines including interleukins, granulocyte macrophage-colony stimulating factor and tumour necrosis factor-α. These can either directly or in conjunction with one another influence growth, differentiation, migration and activation of eosinophils, neutrophils, mast cells, macrophages and lymphocytes. Epithelial cells in atopic and non-atopic individuals are thought to synthesize different amounts and a variety of profiles of cytokines.

9.2 Nasal Biopsy

Written consent was obtained from each patient prior to the procedure and a single nasal biopsy was taken from each patient. Nasal biopsies were obtained from the anterior end of the inferior turbinate, using a technique described by Prior (Prior et al, 1995). The inferior turbinate was decongested and anaesthetized by the application of a pledget of cotton wool soaked in 0.5-0.75 ml of 10% cocaine hydrochloride sterile solution. The pledget was placed between the inferior turbinate and the septum for ten minutes. A biopsy specimen of approximately 2-3mm³ in size was removed from each individual using cup biopsy specimen was immediately placed in ice-cold Medium 199
containing a 1.5% (v/v) antibiotic/antimycotic additive. Each biopsy was processed for tissue culture.
Fig. 13  Human nasal epithelial cell culture

Fig. 14  Laboratory Incubator
9.3  **Culture of human nasal epithelial cells (HNEC’s)**

9.3.1  Culture Media

A number of specially prepared media were used during the culture of HNECs. A description of these media ensues:

1.  **Media 199 plus 1.5% (v/v) antibiotic/antimycotic**

   This solution contained Medium 199 (Northumbria Biological Ltd) plus 10,000 units/ml penicillin; 10mg/ml streptomycin and 25µg/ml amphotericin B (Sigma Chemicals, UK). This solution was used for transporting and washing the biopsy specimens prior to culture.

2.  **Complete Medium 199**

   This was prepared in 500ml aliquots. It consisted of 12.5ml foetal calf serum (Becton Dickinson Ltd, Oxford, UK); 5 ml bovine pancreatic insulin (2.5µg/ml); 5ml L-glutamine (0.02mg/ml); 150µl epidermal growth factor (20ng/ml) and 7.5ml antibiotic/antimycotic solution made up to a final volume of 500ml with Medium 199. This solution was sterilized by filtration through a 0.20µm syringe filter (Sartorius, Milistart, UK) and was used for the culturing of cells during the first 5 days.

3.  **Maintenance Medium 199**

   Maintenance Medium 199 was prepared as described above, however
foetal calf serum was replaced by 12.5ml Nu-Serum IV 2.5% (Universal Biologicals Ltd, UK). This medium was used to sustain the cell culture subsequent to the first 5 days and was changed 3 times weekly there-after.

9.3.2 Culture Technique

HNECs were grown from the biopsy specimens as explant culture, using the technique described by Steele & Arnold and modified by Devalia (Steele and Arnold 1985; Gotfried 2004) (Devalia, J. L., R. J. Sapsford, et al. (1990). "Culture and comparison of human bronchial and nasal epithelial cells in vitro." Respir Med 84(4): 303-12).

The epithelium from each biopsy specimen was carefully dissected away from the underlying lamina propria using a dissecting microscope and cut into smaller sections approximately 1-2mm$^3$ in size.

All sections were washed 3 times in pre-warmed Medium 199 containing 1.5% (v/v) antibiotic/antimycotic solution prior to explantation. Single sections of epithelium were explanted onto 6cm diameter Falcon Primaria culture dishes (Becton Dickinson Ltd, Oxford, UK) with 1.5ml of Complete Medium 199.

The culture dishes were incubated at 37$^\circ$C in a 5% CO$_2$ in air atmosphere at 80-90% humidity (Incubator model Sherlab TC2323). After 5 days incubation in complete Medium 199 the medium was replaced with
Maintenance Medium 199 which was changed 3 times weekly thereafter. The HNECs were allowed to grow to confluence, a process taking 3-4 weeks on average. Once confluence had been attained the explants were removed from the culture dishes and the epithelial cell cultures washed gently with 2mls of freshly prepared Maintenance Medium 199. A further week of incubation was allowed for the epithelial cell cultures to overgrow the area left by the removed explant.

9.3.3 **Confirmation of epithelial cell identity**

9.3.3.1 Light microscopy

All HNEC cultures were examined by phase contrast light microscopy using an Olympus IMT-2 inverted microscope (Olympus Optical, UK), modified with the Hoffman modulation contrast optical system (Modulation Optics Inc, Greenvale, NY, USA). This allowed a detailed 3-D visualisation of the topographical features of the cells and confirmation of epithelial cell identity.

9.4 **Exposure of HNECs to LPS and Macrolides.**

9.4.1 Method

24 hours prior to exposure the HNEC Maintenance Medium 199 was carefully removed with a pipette and replaced by 2mls of Medium 199 plus macrolide plus 1.5% (v/v) antibiotic/antimycotic.
Thirty minutes prior to exposure the HNECs were gently washed 2 times with Medium 199 plus 1.5% (v/v) antibiotic/antimycotic, in order to remove any cytokines generated overnight. Two millimeters of Medium 199 plus 1.5% (v/v) antibiotic/antimycotic were then placed in each dish in order to bathe the cells during the experiment.

HNECs from each patient within the 3 groups were further sub-grouped according to exposure of sample:

1. Control (C)
2. LPS Endotoxin (L)
3. Macrolide- Erythromycin (M)
4. LPS and Macrolide (LM)

9.4.2 Controls
These HNEC’s were not exposed to either macrolide or endotoxin and were assayed to obtain a baseline levels of IL-6 and IL-8 for each patient.

9.4.3 LPS
LPS endotoxin was prepared from E.Coli endotoxin concentrate by routine methods as applied by Khair, Devalia et al. 1994; Khair, Devalia et al. 1995; Khair, Davies et al. 1996. This enabled evaluation of the effect of macrolide exposure to bacterial endotoxin-stimulated HNECs in all three patient groups.

Prior to cytokine measurement the HNECs were placed into the main
Sherlab incubator for a 24 hours. Once the 24 hours had elapsed the culture medium was siphoned off using a pipette and stored at -70°C until analysed for IL-6, and IL-8. The adherent cells were scraped from the culture dish; collected in 1ml of Medium 199 and stored at -70°C until analysed for total cellular protein.

9.4.4 Macrolides

The confluent cultured human nasal epithelial cells were incubated with 10⁻⁵ macrolide (Miyanohara, Ushikai et al. 2000) for 24 hours. The cells were removed from tissue culture plate and used for evaluation of protein concentration. Sets of at least six separate cultures for each specimen were sub-grouped and incubated at 37°C in 5% CO₂ in air atmosphere for 24hrs. The effect of each treatment regimen was investigated on the same day. At the end of incubation, the medium from each culture was stored at -70°C until analysis for IL-6 and IL-8 using commercially available enzyme-linked immunosorbent assay (ELISA) kits (British Biotechnology Ltd, Abingdon, UK). The cells in each culture were collected for protein analysis and the results for the mediators released by HNEC’s were expressed as pg.µg⁻¹ cellular protein.

In order to determine whether or not release of cytokines in HNEC’s is affected by endotoxin or macrolides, at the end of each incubation period, the medium and cells were collected and analysed for IL-6 and
IL-8 total cellular protein as previously described.

The concentration of each mediator present in the medium at the end of incubation was assessed by ELISA ( enzyme-linked immunoassay) as mentioned earlier.

9.5  **Cytokine measurement**

9.5.1  **Method**

Culture media stored at −70°C were allowed to thaw completely prior to Cytokine measurement. The analysis for IL-6 and IL-8 was carried out using commercially available ELISA kits, which were used according to the manufacturer’s instructions (R&D Systems, Abingdon, UK). These assay kits were highly reproducible, specific and sensitive, with no significant cross reactivity or interference between the 3 cytokines investigated. Results for IL-6 and IL-8 were given as pg/ml. In order to account for differences in the number of cytokine producing cells in each culture dish, analysis of the total cellular protein was performed, and cytokine production was therefore expressed as pg of cytokine per µg of cellular protein.

9.6  **Measurement of cellular protein**

9.6.1  **Method**

Measurement of total cellular protein was performed using a modified Lowry
method (Lowry et al, 1951). The technique involved the treatment of proteins with a copper reagent, which reacted with phenolic amino acids to produce a blue colour. The blue colour was then measured spectrophotometrically.

The reagent used were as follows:

Reagent A: 2% Sodium carbonate in 0.1M NaOH
Reagent B: 0.5% Copper sulphate in 1% sodium potassium tartrate
Reagent C: 100 parts of Reagent A: 2 parts of Reagent B
Reagent D: Folin’s and Ciocalteau’s phenol reagent diluted 1.5 times with distilled water

100µl of cellular protein sample was made up to 500µl with 0.15M Saline solution. 2.5 ml of Reagent C was added to each sample using a vortex mixer and allowed to stand for 10 minutes at room temperature.

0.25 ml of Reagent D was the added and mixed immediately and allowed to stand at room temperature for 30 minutes. This reaction resulted in a blue solution which was measured spectrophotometrically at an absorbance value of 758nm, in a Cecil Model CE 292 UV Spectrophotometer (Cecil, Cambridge,UK). This method was capable of detecting total protein concentrations as low as 2µg absolute. The amount of cellular protein per sample was determined from a protein standard curve prepared using serial dilutions of crystalline bovine serum albumin ranging from 0-100 mg/assay. Fresh standards
were prepared for each experiment.
10 EXPERIMENTS
10.1 **Ethics approval**

Full ethics approval for this study was obtained from the East London and City Health Authority Research Ethics Committee. Study No. P/99/194.

10.2 **Subject selection**

A total of 43 patients were recruited into the study. Subjects were characterized into one of three groups.

The groups were as follows:

“Normal subjects” (n=15)

“Allergic rhinitics” (n=12)

“Chronic rhinosinusitics” (n=21)

Patients were initially assessed in the ENT Outpatient clinic at St.Bartholomew’s Hospital, London.

All subjects were:

1. Over 18 years of age.
2. Not taking any anti-allergy medication (within the previous 8 weeks).
3. Not taking any medical treatment known to cause rhinitis.
4. Non-pregnant.

10.3 **Characterisation of Normal subjects.**

**History**- Normal subject had no medical history of atopic or respiratory disease including rhinitis, asthma or COPD. All selected were non-smokers.
Examination- No evidence of rhinitis, asthma or COPD.

Investigations- All subjects were deemed to have normal lung function as measured by spirometry and to be non-atopic as evinced by prick testing. Spirometric measurements were taken under my supervision using a Micro Spirometer. (Micro Medical Ltd, Rochester, UK). Normal values were taken to be FEV₁ > 70% PREDICTED AND FEV₁/FVC ratio > 70%.

The skin prick testing technique is described in 8.3.4.1

10.4 Characterisation of Rhinitics subjects

History- Rhinitic subjects had a positive history for rhinitis as defined by the International Rhinitis Management Working Group (Lund et al, 1994). This requires a minimum of two of the following symptoms for > 1 hour a day on most days.

1. Nasal discharge
2. Nasal blockage
3. Sneeze / Itch

In order to ensure a significant burden of disease in the study patients, the afore-mentioned symptoms must have been present for more than 6 months.

There was no medical history of respiratory disease including asthma or COPD. All Rhinitic subjects were life-long non-smokers.

Examination- No evidence of asthma or COPD.
**Investigations** - All Rhinitic subjects in this study were skin prick test positive to house dust mite allergen only.

All subjects were deemed to have normal lung function as measured by spirometry.

Spirometric measurements were taken under my supervision using a Micro Spirometer. Normal values were taken to be FEV1>70% predicted and FEV1/FVC ratio> 70%.

10.4.1 Skin prick allergy testing.

Prior to skin testing verbal consent was sought from all patients. Patients were asked about precluding factors for skin prick testing, including a history of severe allergic reactions, anaphylaxis, significant eczema or dermatographism. All 43 patients gave their consent and there were no precluding factors.

Test substances used were Soluprick SQ allergen extracts, provided by ALK-Abelló lancet was used to pierce the skin. The lancet is designed to allow penetration of approximately 5 nl of fluid into the subcutaneous layer.

The test substances used in this study were:-

1. Negative control (Saline)
2. Positive control
3. House dust mite (Dermatophagoides pteronyssinus)
4. Five grass mix (Timothy grass, Rye grass, Perennial rye, Meadow fescue, Bermuda grass).

5. Four tree mix (Birsch, Alder, Hazel, Hornbeam)

6. Mould (Aspergillus Fumigatus)

7. Dog (Canis familiaris)

8. Cat (Felix domesticus)

Wheals with a diameter 3mm greater than the negative control, with associated flare and itching were considered as a positive skin prick test reaction.
10.5 Characterisation of Chronic Rhinosinusitic Patients

21 patients with duration of more than 3 months of symptoms compatible with Chronic Rhinosinusitis were recruited to the study group. All patients were ‘skin-prick test’ negative. Symptoms included prolonged nasal congestion or obstruction, thick green-yellowish rhinorrhoea, postnasal drip, anosmia, malaise, attacks of headache, facial pain and discomfort or pressure in the regions of the sinuses. The patients were scheduled for functional endoscopic sinus surgery (FESS) because of insufficient clinical response to repeated antibiotics, mucolytics, antihistamines, decongestants and intranasal steroid sprays.

10.6 Statistical analysis

Statistical analysis was carried out using the computer based analysis program SPSS 15(Statistical Package for Social Science, for PC, SPSS Inc, Chicago IL, USA).

Kolmogorov- Smirnov analysis was used as a test of normality. The Wilcoxon Signed Ranks test was used to compare related samples and the Mann- Whitney test was used to compare independent samples. P values <0.05 were considered to be statistically significant.
11. RESULTS
11. **RESULTS**

The effects of 24 hours exposure to LPS endotoxin, Erythromycin and a combination of both on human epithelial cells cultured from Normal subjects, Allergic Rhinitics and Chronic Rhinosinusitics.

11.1 **Clinical details of subjects**

Nasal epithelial cells (HNECs) were cultured from Normal subjects, Allergic Rhinitics and Rhinosinusitics. Individual clinical details for each group are presented below.

Table 1. Clinical details of Allergic Rhinitic Subjects (n=12)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age/ yrs</th>
<th>Height cm</th>
<th>Weight</th>
<th>BMI kg/m²</th>
<th>FEV1 l</th>
<th>FVC l</th>
<th>PEFR l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>M</td>
<td>30</td>
<td>167</td>
<td>85</td>
<td>30.5</td>
<td>3.2</td>
<td>3.26</td>
<td>595</td>
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<td>A2</td>
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<td>34</td>
<td>173</td>
<td>72</td>
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<td>3.43</td>
<td>3.65</td>
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<td>4.4</td>
<td>590</td>
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<td>430</td>
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Table 2

Clinical details of Normal Subjects (n=15)
Table 3
Clinical details of Chronic Rhinosinusitics (n=21)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>FEV1 (l)</th>
<th>FVC (l)</th>
<th>PEFR (l/min)</th>
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</thead>
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<td>164</td>
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<td>2.98</td>
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<td>3.32</td>
<td>450</td>
</tr>
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</tr>
<tr>
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<td>454</td>
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<td>CRS19</td>
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<td>3.19</td>
<td>3.22</td>
<td>615</td>
</tr>
<tr>
<td>CRS20</td>
<td>F</td>
<td>32</td>
<td>167</td>
<td>90</td>
<td>32.5</td>
<td>3.52</td>
<td>3.61</td>
<td>416</td>
</tr>
<tr>
<td>CRS21</td>
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<td>80</td>
<td>24.5</td>
<td>3.27</td>
<td>3.40</td>
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</table>
Table 4

A comparison of the log^{10} cellular protein concentration of Il-6 and IL-8 in pre- and post stimulated epithelial cells in ‘Normal’ patients (pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>IL6 control</th>
<th>IL8 control</th>
<th>IL6 macrolide</th>
<th>IL8 macrolide</th>
<th>IL6 Stimulated</th>
<th>IL8 Stimulated</th>
<th>IL6 Stimulated and Macrolide</th>
<th>IL8 Stimulated and Macrolide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.56</td>
<td>0.92</td>
<td>0.57</td>
<td>0.86</td>
<td>1.07</td>
<td>0.96</td>
<td>0.64</td>
<td>0.68</td>
</tr>
<tr>
<td>S.D</td>
<td>0.15</td>
<td>0.13</td>
<td>0.28</td>
<td>0.16</td>
<td>0.11</td>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Table 5

A comparison of the log^{10} cellular protein concentration of Il-6 and IL-8 in pre- and post stimulated epithelial cells in ‘Chronic Rhinosinusitic’ patients (pg/µg cellular protein)

<table>
<thead>
<tr>
<th>IL6 control</th>
<th>IL6 stimulated</th>
<th>IL6 Stimulated and Macrolide</th>
<th>IL8 control</th>
<th>IL8 stimulated</th>
<th>IL8 Stimulated and Macrolide</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.65</td>
<td>1.35</td>
<td>0.38</td>
<td>1.21</td>
<td>1.29</td>
<td>1.18</td>
</tr>
<tr>
<td>0.88</td>
<td>1.1</td>
<td>0.72</td>
<td>0.88</td>
<td>1</td>
<td>1.13</td>
</tr>
<tr>
<td>0.91</td>
<td>1.1</td>
<td>0.87</td>
<td>1.11</td>
<td>0.97</td>
<td>1.26</td>
</tr>
<tr>
<td>0.76</td>
<td>1.33</td>
<td>0.54</td>
<td>0.88</td>
<td>0.91</td>
<td>0.8</td>
</tr>
<tr>
<td>0.91</td>
<td>1.03</td>
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<td>0.86</td>
<td>0.69</td>
<td>0.76</td>
</tr>
<tr>
<td>0.62</td>
<td>0.93</td>
<td>0.57</td>
<td>0.61</td>
<td>0.97</td>
<td>0.86</td>
</tr>
<tr>
<td>0.68</td>
<td>1.26</td>
<td>0.58</td>
<td>0.85</td>
<td>0.86</td>
<td>0.91</td>
</tr>
<tr>
<td>0.81</td>
<td>1.17</td>
<td>0.76</td>
<td>0.95</td>
<td>0.91</td>
<td>0.97</td>
</tr>
<tr>
<td>0.79</td>
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<tr>
<td>0.86</td>
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<td>0.79</td>
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<td>0.81</td>
<td>0.49</td>
<td>0.76</td>
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<td>1.11</td>
</tr>
<tr>
<td>0.73</td>
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<td>0.77</td>
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<td>0.93</td>
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<td>0.77</td>
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<td>1.05</td>
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<td>0.18</td>
<td>1.01</td>
<td>1.09</td>
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<td>0.76</td>
<td>1.26</td>
<td>0.79</td>
<td>0.93</td>
<td>1.12</td>
<td>1.21</td>
</tr>
</tbody>
</table>

**MEAN**

| 0.77 | 1.12 | 0.53 | 0.91 | 0.91 | 1.09 | 1.12 | 1.17 |

**S.D**

| 0.11 | 1.12 | 0.2  | 0.16 | 0.18 | 0.15 | 0.11 | 0.09 |
Table 6

A comparison of the log^{10} cellular protein concentrations of IL-6 and IL-8 in pre- and post stimulated epithelial cells in ‘Allergic Rhinitic’ patients (pg/µg cellular protein)

<table>
<thead>
<tr>
<th></th>
<th>IL6 control</th>
<th>IL8 control</th>
<th>IL6 macrolide</th>
<th>IL8 macrolide</th>
<th>IL6 Stimulated</th>
<th>IL8 Stimulated</th>
<th>IL6 Stimulated and Macrolide</th>
<th>IL8 Stimulated and Macrolide</th>
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</thead>
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<tr>
<td>0.63</td>
<td>0.89</td>
<td>0.42</td>
<td>0.58</td>
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<td>0.75</td>
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<table>
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<th></th>
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<th>S.D</th>
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<tr>
<td>0.66</td>
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<td>0.07</td>
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<tr>
<td>0.73</td>
<td>0.97</td>
<td>0.14</td>
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<td>0.39</td>
<td>0.16</td>
<td>0.07</td>
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<td>0.68</td>
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<td>0.1</td>
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11.2 **INTERGROUP STATISTICS**

The results below compare the baseline interleukin activity within the three patient groups. Levels of IL-6 were significantly higher in unstimulated Allergic Rhinitics in comparison with Normal patients (p=0.04). IL-8, however, is lower in Allergics than Normals with Chronic Rhinosinusitis showing the highest levels of activity (p=0.001).

This is shown below in tables 7 and 8.

**Baseline levels of IL-6 and IL-8 in un-stimulated Allergic and Normal Patients**

(\text{pg/\mu g cellular protein})

**Table 7**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated IL6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic</td>
<td>12</td>
<td>0.66</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>0.57</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Unstimulated IL8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic</td>
<td>12</td>
<td>0.73</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>0.92</td>
<td>0.14</td>
<td>0.04</td>
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</table>

**Table 8**

<table>
<thead>
<tr>
<th></th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated IL6</td>
<td>Equal variances assumed</td>
<td>0.044</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Unstimulated IL8</td>
<td>Equal variances assumed</td>
<td>0.001</td>
<td>-0.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Macrolides lower IL-6 and IL-8 in unstimulated HNEC’s in all three groups however the response is more prevalent in AR (tables 11-16).

**Effect of macrolides on baseline levels of IL-6 in Allergic and Normal Patients**

**(pg/µg cellular protein)**

<table>
<thead>
<tr>
<th></th>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control IL6</strong></td>
<td>Allergic</td>
<td>12</td>
<td>0.66</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>15</td>
<td>0.57</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Macrolide IL6</strong></td>
<td>Allergic</td>
<td>12</td>
<td>0.39</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>15</td>
<td>0.57</td>
<td>0.28</td>
<td>0.07</td>
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</table>

**Table 10**  
**Independent Samples Test**

<table>
<thead>
<tr>
<th></th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control IL6</strong></td>
<td>Equal variances assumed</td>
<td>0.044</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Macrolide IL6</strong></td>
<td>Equal variances assumed</td>
<td>0.064</td>
<td>-0.17</td>
<td>0.09</td>
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</table>
The effect of macrolides on IL-8 in pre and post stimulated cells of Allergic and Normal patients (pg/µg cellular protein)

Table 11

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated IL8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergics</td>
<td>12</td>
<td>0.73</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Normals</td>
<td>15</td>
<td>0.92</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Macrolide IL8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergics</td>
<td>12</td>
<td>0.5</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Normals</td>
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<td>0.86</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Stimulated IL8</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Allergics</td>
<td>12</td>
<td>1.01</td>
<td>0.28</td>
<td>0.08</td>
</tr>
<tr>
<td>Normals</td>
<td>15</td>
<td>0.96</td>
<td>0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>Stimulated and macrolide IL8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergics</td>
<td>12</td>
<td>0.68</td>
<td>0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Normals</td>
<td>15</td>
<td>0.68</td>
<td>0.12</td>
<td>0.03</td>
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</table>

Table 12

<table>
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<th>Equal variances assumed</th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control IL8</td>
<td>0.001</td>
<td>-0.2</td>
<td>0.05</td>
<td>-0.29, -1</td>
</tr>
<tr>
<td>Macrolides IL8</td>
<td>0.001</td>
<td>-0.37</td>
<td>0.06</td>
<td>-0.48, -0.25</td>
</tr>
<tr>
<td>Stimulated IL8</td>
<td>0.5</td>
<td>0.06</td>
<td>0.1</td>
<td>-0.15, 0.27</td>
</tr>
<tr>
<td>Stimulated and Macrolides IL8</td>
<td>0.91</td>
<td>0.01</td>
<td>0.04</td>
<td>-0.08, 0.09</td>
</tr>
</tbody>
</table>
Effect of macrolides on baseline levels of IL-6 in Chronic Rhinosinusitis and Normal patients (pg/µg cellular protein)

Table 13

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6 Unstimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CRS</td>
<td>21</td>
<td>0.78</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>0.57</td>
<td>0.11</td>
<td>0.029</td>
</tr>
<tr>
<td>IL6 Stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRS</td>
<td>21</td>
<td>0.91</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>1.1</td>
<td>0.11</td>
<td>0.028</td>
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<tr>
<td>IL6 Stimulated and macrolide</td>
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<td></td>
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<tr>
<td>CRS</td>
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<td>0.11</td>
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Table 14

Independent Samples Test

<table>
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<th></th>
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<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
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</thead>
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<tr>
<td></td>
<td></td>
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<tr>
<td>IL6 Unstimulated</td>
<td>0.001</td>
<td>-0.21</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>Equal variances</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>assumed</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL6 Stimulated</td>
<td>0.004</td>
<td>-0.17</td>
<td>0.05</td>
<td>-0.28</td>
</tr>
<tr>
<td>Equal variances</td>
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<td></td>
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</tr>
<tr>
<td>assumed</td>
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<tr>
<td>IL6 Stimulated and macrolide</td>
<td>0.001</td>
<td>0.47</td>
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<td>0.39</td>
</tr>
<tr>
<td>Equal variances</td>
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<td></td>
</tr>
<tr>
<td>assumed</td>
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</tbody>
</table>
Effect of macrolides on baseline levels of IL-8 in Chronic Rhinosinusitics and Normal patients (pg/µg cellular protein)

Table 15

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8 Unstimulated</td>
<td>CRS</td>
<td>21</td>
<td>0.91</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>15</td>
<td>0.86</td>
<td>0.16</td>
</tr>
<tr>
<td>IL8 Stimulated</td>
<td>CRS</td>
<td>21</td>
<td>1.1</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>15</td>
<td>0.96</td>
<td>0.25</td>
</tr>
<tr>
<td>IL8 Stimulated and macrolide</td>
<td>CRS</td>
<td>21</td>
<td>1.17</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
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<td>0.68</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 16

Independent Samples Test

<table>
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<tr>
<th></th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8 Unstimulated</td>
<td>Equal variances assumed</td>
<td>0.39</td>
<td>0.06</td>
<td>-0.06</td>
</tr>
<tr>
<td>IL8 Stimulated</td>
<td>Equal variances assumed</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.008</td>
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<tr>
<td>IL8 Stimulated and macrolides</td>
<td>Equal variances assumed</td>
<td>0.001</td>
<td>0.03</td>
<td>0.42</td>
</tr>
</tbody>
</table>
11.3 INTRA-GROUP ANALYSIS

11.3.1 Normals

- Baseline IL-6 levels are lower than AR and CRS (p=0.04)
- Baseline IL-8 levels are lower than CRS but not AR (p=0.001)
- LPS raises IL-6 (p=0.001) and mildly raises IL-8 (p=0.57)
- Macrolides markedly lower IL-6 (p=0.001) and IL-8 (p=0.001) in stimulated cells

A comparison of the effect of macrolides on stimulated and unstimulated IL6 and IL-8 in Normal patients (pg/µg cellular protein)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6 Control</td>
<td>0.5684</td>
<td>15</td>
<td>0.11387</td>
<td>0.0294</td>
</tr>
<tr>
<td>IL6 Stimulated</td>
<td>1.0729</td>
<td>15</td>
<td>0.10887</td>
<td>0.02811</td>
</tr>
<tr>
<td>Pair 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8 Control</td>
<td>0.922</td>
<td>15</td>
<td>0.13882</td>
<td>0.03584</td>
</tr>
<tr>
<td>IL8 Stimulated</td>
<td>0.9584</td>
<td>15</td>
<td>0.25015</td>
<td>0.06459</td>
</tr>
<tr>
<td>Pair 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6 Stimulated</td>
<td>1.0729</td>
<td>15</td>
<td>0.10887</td>
<td>0.02811</td>
</tr>
<tr>
<td>IL6 Stimulated and macrolide</td>
<td>0.6534</td>
<td>15</td>
<td>0.12017</td>
<td>0.03103</td>
</tr>
<tr>
<td>Pair 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8 Stimulated</td>
<td>0.9584</td>
<td>15</td>
<td>0.25015</td>
<td>0.06459</td>
</tr>
<tr>
<td>IL8 Stimulated and macrolide</td>
<td>0.6765</td>
<td>15</td>
<td>0.11523</td>
<td>0.02975</td>
</tr>
</tbody>
</table>
11.3.2 Chronic Rhinosinusitics

- Baseline IL-6 higher than Normals and AR (p=0.04)
- Baseline IL-8 higher than Normals and AR (p=0.001)
- LPS increases IL-6 (p=0.02) however has little effect on IL-8 levels (p=0.48)
- Macrolides increase IL-6 (p=0.001) and IL-8 (p=0.02) in stimulated cells
- Macrolides decrease IL-6 (p=0.001) and IL-8 (p=0.001) in unstimulated cells

A comparison of the effect of macrolides on stimulated and unstimulated IL-6 and IL-8 in Chronic Rhinosinusitic patients (pg/µg cellular protein)

### Table 18

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>Sig.(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>IL6 Pre and post stimulation</td>
<td>-0.5</td>
<td>0.04</td>
<td>-0.6</td>
<td>-0.42</td>
</tr>
<tr>
<td>Pair 2</td>
<td>IL6 Pre and post stimulation</td>
<td>-0.04</td>
<td>0.06</td>
<td>-0.17</td>
<td>0.1</td>
</tr>
<tr>
<td>Pair 3</td>
<td>IL6 Stimulated and macrolide</td>
<td>0.42</td>
<td>0.05</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td>Pair 4</td>
<td>IL6 Stimulated and macrolide</td>
<td>0.28</td>
<td>0.06</td>
<td>0.16</td>
<td>0.41</td>
</tr>
</tbody>
</table>

### Table 19

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>N</th>
<th>Std Deviation</th>
<th>Std Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>IL6 Unstimulated</td>
<td>0.77</td>
<td>21</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>IL6 Stimulated</td>
<td>0.91</td>
<td>21</td>
<td>0.18</td>
</tr>
<tr>
<td>Pair 2</td>
<td>IL8 Unstimulated</td>
<td>1.12</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>IL8 Stimulated</td>
<td>1.09</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td>Pair 3</td>
<td>IL8 Stimulated</td>
<td>0.91</td>
<td>21</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>IL8 Stimulated and macrolide</td>
<td>1.12</td>
<td>21</td>
<td>0.12</td>
</tr>
<tr>
<td>Pair 4</td>
<td>IL8 Stimulated</td>
<td>1.1</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>IL8 Stimulated and macrolide</td>
<td>1.17</td>
<td>21</td>
<td>0.09</td>
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</tbody>
</table>
### Table 20 Paired Samples Statistics

<table>
<thead>
<tr>
<th>Pair</th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>Sig.(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Pair 1</td>
<td>IL6 Pre and post stimulation</td>
<td>-0.13</td>
<td>0.052</td>
<td>-0.24</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>IL8 Pre and post stimulation</td>
<td>0.03</td>
<td>0.04</td>
<td>-0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Pair 2</td>
<td>IL6 Stimulated and macrolide</td>
<td>-0.214</td>
<td>0.044</td>
<td>-0.31</td>
<td>-0.12</td>
</tr>
<tr>
<td></td>
<td>IL8 Stimulated and macrolide</td>
<td>-0.09</td>
<td>0.03</td>
<td>-0.15</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

### The effect of macrolides on baseline IL-6 and IL-8 levels in Chronic Rhinosinusitis (pg/µg cellular protein)

### Table 21

<table>
<thead>
<tr>
<th>Pair</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6 Unstimulated</td>
<td>0.78</td>
<td>21</td>
<td>0.11</td>
<td>0.03</td>
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<tr>
<td>IL6 Macrolide</td>
<td>0.53</td>
<td>21</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>IL8 Unstimulated</td>
<td>1.12</td>
<td>21</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>IL8 Macrolide</td>
<td>0.91</td>
<td>21</td>
<td>0.16</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Table 22

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>Sig.(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair 1 IL6 Unstimulated and macrolide</td>
<td>0.25</td>
<td>0.45</td>
<td>0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>Pair 2 IL8 Unstimulated and macrolides</td>
<td>0.21</td>
<td>0.19</td>
<td>0.12</td>
<td>0.29</td>
</tr>
</tbody>
</table>

11.3.3 Allergics

- Baseline IL-6 higher than in Normal (p=0.04)
- Baseline IL-8 lower than in Allergics (p=0.001)
- LPS significantly increases IL-6 (p=0.02) and IL-8 (p=0.01) levels
- Macrolides lower IL-6 (p=0.001) and IL-8 (p=0.001) levels in stimulated and unstimulated cells

A comparison of the effect of macrolides on stimulated and unstimulated IL-6 and IL-8 in Allergic Rhinitic patients (pg/µg cellular protein)

Table 23

<table>
<thead>
<tr>
<th>Paired Samples Statistics</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1 IL6 pre and post-stimulated</td>
<td>0.66</td>
<td>12</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>12</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Pair 2 IL8 pre and post-stimulated</td>
<td>0.73</td>
<td>12</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>12</td>
<td>0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Pair 3 IL6 stimulated and macrolide</td>
<td>0.73</td>
<td>12</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>12</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Pair 4 IL8 stimulated and macrolide</td>
<td>1.01</td>
<td>12</td>
<td>0.28</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>12</td>
<td>0.1</td>
<td>0.03</td>
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<tr>
<td>Pair 5 IL6 Unstimulated and macrolide</td>
<td>0.66</td>
<td>12</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.39</td>
<td>12</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>Pair 6 IL8 Unstimulated and macrolide</td>
<td>0.73</td>
<td>12</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>12</td>
<td>0.12</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Table 24

Paired Samples Test

<table>
<thead>
<tr>
<th>Pair</th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval of the Difference</th>
<th>Sig.(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL6 Pre and post stimulation</td>
<td>-0.07</td>
<td>0.03</td>
<td>-0.13</td>
<td>-0.01</td>
</tr>
<tr>
<td>2</td>
<td>IL8 Pre and post stimulation</td>
<td>-0.29</td>
<td>0.09</td>
<td>-0.48</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>IL6 Stimulated and macrolide</td>
<td>0.13</td>
<td>0.04</td>
<td>0.26</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>IL8 Stimulated and macrolide</td>
<td>0.33</td>
<td>0.09</td>
<td>0.13</td>
<td>0.54</td>
</tr>
<tr>
<td>5</td>
<td>IL6 Unstimulated and macrolide</td>
<td>0.27</td>
<td>0.04</td>
<td>0.18</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td>IL8 Unstimulated and macrolide</td>
<td>0.23</td>
<td>0.04</td>
<td>0.13</td>
<td>0.33</td>
</tr>
</tbody>
</table>
The results are further demonstrated graphically below in Figures 17, 18, 19, 20, 21, 22, 23, 24, 25 & 26

**Baseline IL-6 release from each of the 3 patient groups**

**Fig. 17**

![IL-6 Graph](image)

*IL-6*

**IL-6**

- **ALLERGIC**
- **CRS GROUP**
- **NORMALS**

**IL6**

95%

C.I

P_g/µg

\[ p=0.044 \]
Baseline IL-8 release from each of the 3 patient groups

Fig. 18

IL-8

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ALLERGICS</th>
<th>CRS</th>
<th>NORMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean IL8 +/− 2S.D</td>
<td>0.80</td>
<td>1.00</td>
<td>1.20</td>
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</tbody>
</table>

P=0.001
IL-6 RELEASE IN STIMULATED AND UN-STIMULATED HNEC’S IN THE THREE GROUPS- Pg/µg

Fig. 19

ALLERGICS  CRS  NORMAL

GROUP

95 % CI

control  stimulated  control  stimulated  control  stimulated
IL-8 RELEASE IN STIMULATED AND UNSTIMULATED HNEC’S IN THE THREE GROUPS-Pg/µg

Fig. 20

ALLERGICS  CRS  NORMALS

GROUP

1

2

3

95 % CI

CONTROL  STIMULATED  CONTROL  STIMULATED  CONTROL  STIMULATED

1.2

1.1

1.0

0.9

0.8

0.7

0.6
The effect of 24 hour macrolide exposure on IL-6 release in stimulated HNECs of the 3 patient groups

Fig. 21

![Graph showing IL-6 release with 95% CI for each group and significance levels: p=0.01, p=0.02, p=0.001 for Allergics, CRS, and Normals, respectively.](image-url)
The effect of 24 hour macrolide exposure on IL-8 release in stimulated HNECs of the 3 patient groups

Fig. 22

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ALLERGICS</th>
<th>CRS</th>
<th>NORMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8 95% C.I.</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>STIMULATED MACROLIDES</td>
<td>STIMULATED MACROLIDES</td>
<td>STIMULATED MACROLIDES</td>
<td></td>
</tr>
</tbody>
</table>

p=0.01  p=0.001  p=0.001
11.4 Summary of results

Allergic Rhinitics
Baseline levels of IL-6 in un-stimulated Allergic Rhinitics are significantly higher than in Normal patients. This indicates a pre-existing increased level of cytokine activity within nasal epithelium in un-stimulated Allergic Rhinitics. Baseline levels of IL-8, however, are lowest in Allergics. LPS significantly stimulates this group to increase production of both IL-6 and IL-8 LPS significantly raises IL-6 and IL-8 in AR. Macrolides lower IL-6 and IL-8 in both stimulated and unstimulated cells.

Chronic Rhinosinusitics
The results show baseline levels of IL-6 and IL-8 are higher in CRS than AR and Normals. This may be explained by increased neutrophilic and eosinophilic activity in CRS as opposed to AR. LPS stimulates IL-6 in CRS. LPS significantly raises IL-6 and IL-8 in CRS.

Macrolides increase IL-6 and IL-8 in stimulated cells however reduce levels of both in un-stimulated cells. These results are observed at 24 hours following exposure to macrolide.

Normals
Baseline levels of IL-6 and IL-8 are lower in Normals than AR or CRS. LPS significantly raised IL-6 however had little effect on IL-8 levels.
Macrolides lowered IL-6 and IL-8 in stimulated cells.
12. DISCUSSION
12. DISCUSSION

The term ‘rhinitis’ implies inflammation. The symptoms include sneezing, irritation, anterior discharge, hyposmia, anosmia and nasal obstruction. Secondary symptoms include headache, facial pain, ear popping, nasal obstruction, dry throat, post-nasal drip, cough and eye symptoms. It is estimated that morbidity associated with nasal symptoms is responsible for over £1.5million caused by loss of working hours. The pathophysiology of rhinitis, allergic and non-allergic, is yet to be fully understood. The classification of rhinitis is evolving.

Nasal epithelial cells hold two distinct roles in the inflammatory response. They enable clearance of particulate matter from the airway and also act as a physical barrier to the entry of noxious agents. Recent studies have shown that nasal epithelial cells play a pivotal role in the initiation and control of the inflammatory process. They are able to release biologically active mediators which modulate the function of other inflammatory cells implicated in the pathogenesis of rhinitis. Pro-inflammatory cytokines are of particular interest in allergic rhinitis as they influence the activity of immunocytes such as eosinophils, neutrophils, T-lymphocytes and mast cells. Their presence is a key feature of rhinitis. Studies in vivo and in vitro have shown that HNECs generate a wide variety of cytokines including IL1β, TNFα, IL-6, IL-5, IL-8, GM-CSF, RANTES AND MCP-1 (Devalia, Campbell et al. 1993; Kenney, Baker et al. 1994; Davies, Wang et al.)
1995; Mullol, Xaubet et al. 1995). IL-1β, TNF-α, IL-5 and IL-6 are multifunctional cytokines synthesized and released by airway epithelial cells. They all have pro-inflammatory effects on a variety of target cells. IL6, IL8, TNF-alpha and ICAM-1 have been shown to develop enhanced expression as a result of bacterial endotoxins. Khair et al in 1995 studied the effects of erythromycin on the release of IL6, IL8 and sICAM-1 following haemophilus influenza endotoxin stimulation. Their results showed an increase in neutrophil chemotaxis within cultured human endothelial cells. The airway epithelium is an important barrier between the environment and sub-epithelial tissues. The epithelium is functionally divided into apical and basolateral parts. The elements of the barrier determine the restriction it provides. The protease-activated receptor is expressed in airway epithelium and its activation initiates multiple effects including enhanced airway inflammation reactivity. The effects of protease-activated receptor-2 (PAR-2) stimulation on inflammation mechanisms of chronic rhinosinusitis (CRS) are still unknown. Rudack et al investigated the expression of PAR-2 receptor expression (Rudack, C., M. Steinhoff, et al. (2007). "PAR-2 activation regulates IL-8 and GRO-alpha synthesis by NF-kappaB, but not RANTES, IL-6, eotaxin or TARC expression in nasal epithelium." Clin Exp Allergy 37(7): 1009-22).

In primary nasal epithelial cell cultures, the function of PAR-2 and its ability to
produce chemokines and IL-6 were measured by calcium mobilization and stimulation tests. Inhibition tests were performed using cortisone, serine protease inhibitors, cysteine protease inhibitors. Signal transduction pathways were analysed by electromobility shift assays (EMSA) and NF-kappa B binding studies. The expression of PAR-2 was found to be increased in CRS specimens. The activation of PAR by trypsin or PAR-2-specific activating peptide (AP) caused an increase in cytosolic calcium, as well as the release of the CXC chemokines IL-8 and growth-related oncogene (GRO)-alpha, but not the release of CC chemokines or IL-6. The study concludes that PAR-2 plays a role in protease-mediated regulation - staphylococcal and non-staphylococcal origin - of IL-8 and GRO-alpha in nasal epithelial cells, but not in the regulation of CC chemokines. PAR-2 may therefore be involved in the pathophysiology of CRS and NP at different sites of activation.

The immunomodulatory apparatus that links both chronic rhinosinusitis and allergic rhinitis is complex. An improved understanding of the pathways that are involved is necessary to improve management. The ARIA Guidelines published in 2008 provide an evidence-based guide to the management of allergic rhinitis. The European Position Paper on Rhinosinusitis and Nasal Polyps (2007) provides an evidence-based management scheme for chronic and acute presentations of the condition.

The fundamentals of this study are based on knowledge of the underlying
immunomodulatory pathways and factors established in recent years with both allergic rhinitis and chronic rhinosinusitis. The study aims to identify activity of specific cytokines, IL-6 and IL-8, both of which contribute to pathways of intracellular activity in each condition. In particular the effects of macrolides on both allergic rhinitis and chronic rhinosinusitis can further our understanding of the similarities and differences between the two.

12.1 Culture of HNECs from nasal turbinate biopsy tissue

12.2 The ‘Devalia’ explant technique for culture of HNECs

The ‘Devalia’ explant technique was used to grow HNECs for my experiments. This technique was originally described by Steele and Arnold in 1985 for tissue culture of human bronchial tissue taken from bronchoscopic biopsies (Steele & Arnold, 1985). A modified and highly reproducible method was later developed in our laboratories at St. Bartholomew’s Hospital by Devalia et al, which has subsequently been used for both bronchial and nasal explant cultures (Devalia et al, 1990).

Calderon was the first to study HNECs cultures grown using ‘Devalia’ explant technique. He used electron microscopic and immunohistocytochemical evaluation to confirm that the cultured cells retained their morphological and histochemical characteristics. In addition, light microscopy confirmed normal, synchronous ciliary beat frequency (Calderon et al, 1997). These in vitro functional and
structural characteristics would suggest that ‘Devalia’ nasal explant cultures provide a good in-vitro biological model for the study of rhinitis.

A potential criticism of the ‘Devalia’ explant technique is that contamination of the explants with other cell types can occur, particularly fibroblasts. This problem is tackled by the employment of a number of precautions—

a. Avoidance of deep turbinate biopsies which may inadvertently pick up submucosal cells.

b. Careful dissection of each specimen to remove the underlying lamina propria.

c. The use of Falcon Primaria culture dishes, which are coated with a fibroblast inhibitor

d. Light microscopic examination and disposal of contaminated dishes

12.3 Constitutive cytokine release

Both in vivo and in vitro studies have demonstrated that HNECs can generate a wide variety of cytokines including IL1β, TNFα, IL-5, IL-6, IL-8, GM-CSF, RANTES, MCP-1 and sICAM-1 (Devalia et al, 1993a+b; Davies & Devalia, 1992; Mullol et al, 1995; Kenney et al, 1994; Marini et al, 1992; Salvi et al, 1999; Mills et al, 1999). These cytokines, either directly or in conjunction with one another, influence the growth, differentiation, activation, migration and survival or inflammatory cells.
The two cytokines IL-6 and IL-8 were chosen for the investigation in this study because their exact role in the nasal inflammatory process is unclear and because these cytokines have been exclusively investigated in previous studies from our laboratories, particularly in relation to lower airway inflammation (Khair et al., 1994; Calderon et al., 1997; Mills et al., 1998a; Rusznak et al., 2000). As outlined below IL-6 is released by nasal epithelial cells relatively early in the rhinitic process and acts mainly upon the immediate hypersensitivity response; IL-8 is involved in the late phase response.

Interleukin-6

IL-6 form the group of multi functional cytokines which include IL1-β, TNFα and IL-5 (Borish & Rosenwasser, 1996; Levine, 1995). IL-6 involved in the activation of B lymphocytes and facilitates the switching of plasma cells to produce IgE, thus sensitising the nasal epithelium. IL-6 is also important in the induction of the acute phase response, it produces fever and stimulates hepatocytes to produce acute phase proteins such as fibrinogen, C-reactive protein and serum amyloid A (Castell et al., 1988; Kishimoto et al., 1992).

Interleukin-8

IL-8 is from the group of chemotactic cytokines which include GM-CSF, RANTES and MCP-1. Airway epithelial cells have demonstrated to produce large amounts of IL-8, which on a molar basis is one of the most potent neutrophil
chemo-attractants (Cromwell et al, 1992; Devalia et al, 1993b; Levine, 1995). IL-8 also induces T lymphocyte and eosinophil chemotaxis as well as activating these cells (Baggiolini et al, 1989; Baggiolini, 1992; Borish & Rosenwasser 1996; Shute, 1994; Warringa et al, 1991). It is noteworthy that IL-8 is an extremely stable protein which maintains its biological activity even in the presence of significant changes in pH and proteolytic enzymes, suggesting that once produced it may exert a prolonged biological effect (Baggiolini & Clark-Lewis, 1992).

My results have demonstrated that HNEC explant culture from Normal subjects, Chronic Rhinosinusitis and Allergic Rhinitis are capable of constitutive release of the cytokines IL-6 and IL-8.

Kenny et al (Kenny et al, 1994), demonstrated a similar relationship between IL-8 and IL-6 when they reported constitutive release of cytokines from HNECs as follows:- IL-8 > IL-6 > IL-1α > IL-1β

Similarly Bachert et al (Bachert et al, 1995 a +b), measured cytokine levels in nasal lavage fluid and demonstrated the following relationship- IL-8 > IL-6 > >TNFα > IL-1β

Our studies have demonstrated that IL-6 and IL-8 activity exists in all three groups of subjects. IL-6 and IL-8 levels are highest in Chronic Rhinosinusitis. Pre-existing neutrophilic and eosinophilic activity in CRS subjects may explain the increased baseline levels of both cytokines. IL-8 levels were lowest in AR.
LPS significantly enhanced cytokine activity in all three groups. LPS had little effect on IL-8 in Normals. This study reveals inhibitory activity of Erthromycin in stimulated Allergic Rhinitics and Normal subjects with a reduction in levels of IL-6 and IL-8 seen in both. Stimulated HNEC’s in AR subjects appear to be sensitive to an immunomodulatory effect secondary to erythromycin. AR subjects also showed sensitivity to erythromycin in un-stimulated HNEC’s.

The study identifies a significant difference in activity in HNEC’s of CRS subjects. An increase in IL-6 and IL-8 levels was identified in stimulated CRS, following macrolide exposure, with a contrasting reduction in levels of both IL-6 and IL-8 in unstimulated HNEC’s.

These findings contradict our current understanding behind the principles of macrolide activity. The complexity of the pathophysiology related to individual cytokine function is evident. We can stipulate that cytokine activity in CRS, is not optimally stimulated, and therefore is sensitive to stimulation with LPS. There possible explanations for this response are vast.

An agonistic response mounted by macrolides can be secondary to stimulation of Protease-Activated Receptors that in-turn upregulate IL-6 and IL-8 activity. LPS is known to effect protein expression by IL-8 mRNAs. Macrolides reduce IL-6 and IL-8 levels in un-stimulated chronic rhinosinusitis. This indicates an interplay between macrolides and that altered cytokine activity is directly related to the effects of LPS upon cellular immunomodulation.
The results impress the need for further studies to validate these findings. Saturation of cytokine activity may prevent enhancement of further activity. The study has shown that LPS endotoxin attenuates the release of both inflammatory mediators in AR patients and a significant reduction in IL-6 and IL-8 is shown with exposure to macrolides. The reduction can be explained by the theory that macrolide reduces LPS endotoxin induced chemotaxis and adhesion to human endothelial cells in vitro. This would, in turn, suggest that the effect of LPS endotoxin on mediator release from epithelial cells is a specific effect, and that erythromycin is likely to interfere with this effect. Whilst it is possible that erythromycin may act either to inhibit the expression or the release of these inflammatory mediators from human nasal epithelial cells, it is not possible to determine which of these two processes is affected from the present studies. In order to further investigate the specific mechanism through which erythromycin may operate, we require further studies to investigate the effect of erythromycin on LPS endotoxin – induced changes in the concentration of specific messenger ribonucleic acid transcribed for the mediators investigated in the present study.

Clinical in-vivo studies have already highlighted the relevance of macrolide in the management of chronic rhinosinusitis. Ragab et al have highlighted the long-term use of macrolides as a part of a treatment regimen in chronic rhinosinusitis. The study found significant improvements in both subjective and objective parameters both medical and surgical management of these patients. The aims of this study
were to conduct the first prospective, randomised, controlled trial evaluating and comparing treatment, both surgical and medical in polypoid and no-polypoid chronic rhinosinusitis. A trial of ninety patients concluded a three month course of medical treatment is appropriate prior to considering surgery which should be reserved in refractory cases. More relevant to our work the study concluded a three month course of macrolides be used in the treatment of moderate to severe chronic rhinosinustis.

In this study a concentration of LPS endotoxin and erythromycin were selected on the basis of previous studies which have demonstrated maximal cytokine release from epithelial cell cultures. Although clinical effectiveness of erythromycin in the management of chronic rhinosinusitis has been suitably established, studies of underlying the effects of erythromycin remain wholly unclear. Whilst some studies have suggested this drug acts as an antimicrobial others have suggested that it may have anti-inflammatory effects. Reviews of the mechanisms underlying the antibacterial role of erythromycin and other macrolide antibiotics have demonstrated that these agents exhibit their antimicrobial activity by interfering with protein synthesis in the microorganism (Brisson-Noel et al). These authors suggested that the macrolide antibiotics act primarily by binding reversibly to the 50 S ribosomal sub-units of sensitive microorganisms, and consequently stimulate the dissociation of the peptidyl-transfer ribonucleic acid (tRNA), ribosomes during translocation to the mRNA, rather than preventing the peptide bond.
This study shows a significant anti-inflammatory effect of erythromycin on allergic rhinitics. This has not been previously either studied in vitro or in vivo. It does necessitate further investigation to approach the possible role of macrolides in a purely anti-inflammatory role. Previous studies have demonstrated the effects of erythromycin in different model systems. Iino et al investigated the effect of LPS-induced release of TNF-α from human monocytes, and demonstrated that this was significantly reduced by treatment with erythromycin. Incubation of LPS-stimulated monocytes with non-macrolide drugs such as ofloxacin or penicillin G did not have any effect on TNF-α release. Katoda et al investigated the effect of 4 weeks of treatment with oral erythromycin on neutrophil chemotactic activity (NCA) and neutrophil accumulation in bronchoalveolar lavage (BAL) fluid of patients with diffuse panbronchiolitis, and demonstrated that these were significantly reduced. Anderson et al has suggested that erythromycin may exhibit anti-inflammatory effects inhibiting the generation of superoxide by activated neutrophils.

In summary this study suggests erythromycin exhibits anti-inflammatory effects in Chronic Rhinosinusitics and stimulated Allergic Rhinitics. It is possible that erythromycin could exert its effects by directly inhibiting the activity of inflammatory cells in vivo. My study suggests that this agent is more likely to act indirectly by modulating the synthesis and/or release of pro-inflammatory mediators, such as IL-8 and IL-6 which effect activity of neutrophils, the key
effector cell in the pathogenesis of intermittently exacerbated bacterial infections.

The potential anti-inflammatory effect of macrolides has generated considerable interest in the past decade. The effect on mediators of neutrophil inflammation has been well defined. In contrast the effect of macrolide on eosinophilic chronic rhinosinusitis is still not clear. Macrolides, however, have been in use in the management of chronic rhinosinusitis for some years. This study does encourage this application. Allergic rhinitis also triggers a systemic increase of inflammatory elements. The management of this remains more complex and often poor in compliance as patients find little benefit from the recommended medical treatment and advice. In addition to causing symptoms of rhinitis, inflammatory cells and mediators travel through the circulatory system and are able to target other susceptible tissues, leading to exacerbation of co-morbid conditions such as asthma and sinusitis.

Treatment for Allergic Rhinitis needs to be effective long-term. This study highlights the need for treatment to be directed at its underlying pathophysiological pathway as opposed to simple symptomatic relief. The findings from this are novel and encouraging in the progress of further understanding the effects of macrolides in a potentially pivotal role.
13. **FUTURE STUDIES**
13. FUTURE STUDIES

Epithelial cells are situated, anatomically, in ‘front-line’ and constitute a biochemical interface between the cold, dry, polluted external environment and the internal milieu. Chronic Rhinosinusitics and Rhinitics are two large groups of individuals who suffer from frequent nasal morbidity. In-depth investigation of the mechanisms involved in rhinitis would be incomplete without careful study of these two groups, however, a search of the world literature reveals that cytokine release from HNECs cultured from allergic rhinitis patients and chronic rhinosinusitic has never been compared, (from the point of view of constitutive cytokine release). Furthermore analyses of in-vivo effects of cytokine release in chronic rhinosinusitis have improved our understanding of the mechanism and potential management of this condition.

This study has produced interesting novel data, which has given further insight into mechanisms involved in the nasal inflammatory response. It has, however, raised many interesting questions.

Macrolides are effective in significantly reducing IL-6 and IL-8 activity in stimulated and unstimulated Allergic Rhinitic patients. Cytokine release between individuals may be a consequence of inherent differences in the signal transduction pathways for specific cytokines. The signalling is part of a complex of pathways involving subfamilies of enzymatic proteins such as G- proteins and protein kinases.
Recent studies have focused on the activity of protease-activated receptors. These receptors contribute to the release of pro- and anti-inflammatory mediators. LPS is understood to specifically up-regulate expression of PAR-2 but not PAR-1. Activation of PAR-2, not PAR-1, appears to influence IL-8 production from respiratory epithelial cells. There is significant overlap in the pathways upon which agonists specific to these receptors take effect. The in-vitro studies, thus far, have focused on pathways within the lower respiratory tract. 60% of asthmatics suffer with allergic rhinitis and 20-30% of patients with allergic rhinitis suffer with asthma. In normal subjects it is understood that the structure of the airway mucosa of the nose and bronchi share similarities. It is understood that inflammation has a role in both asthma and rhinitis at a cellular and molecular level. This study should act as a springboard for further research in the upper respiratory tract. Progress could be made by analysing the effects of agonists upon these receptors and thereby the impact on production cytokines such as IL-8; work that has already been carried out on lower respiratory tract epithelium. The impact and expression of LPS and agonists on PAR-1 and PAR-2 in allergic rhinitis would compare immunomodulatory activity within the lung. The quantitative effect of stimulation of these receptors within nasal epithelium and their impact upon sub-groups of interleukins is essential.

Is the anti-inflammatory effect of macrolides altered by an infective stimulatory environment? Perhaps cytokines and their susceptibility to anti-inflammatory
effects of macrolides is reliant upon an optimal level of activity. Chronic Rhinosinusitis may provide these ideal conditions.

In-vivo studies are ultimately required to establish the impact and efficacy of macrolides as a recognised part of the treatment regimen in Allergic Rhinitis as is established in Chronic Rhinosinusitis. These studies would require considerable prior, in-vitro, quantitative analyses of the affect of macrolides on this sub-group of patients.

In summary, it would be reasonable to suggest that differences in cytokine release between individual pathologies result in differences within cytokine formation, storage and release. The impact of further studies should, over time, relate to substantive enhancement of knowledge of a condition that remains often very poorly managed.
14. REFERENCES
REFERENCES


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