Occasional review

Mechanisms of virus induced exacerbations of asthma

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Most work on the pathogenesis of asthma has studied airway inflammation using allergen challenge as a model.¹ Although appropriate for our understanding of the underlying disease pathology, epidemiological studies have suggested that allergens are not common precipitants of asthma attacks^{2,3} and cohort studies have demonstrated the importance of upper respiratory tract (URT) viruses as probably the commonest cause of asthma exacerbations.^{4,5} Studying the effects of URT viruses should therefore help our understanding of the mechanisms underlying exacerbations of asthma and open up new avenues for therapeutic intervention.⁶ In this review we shall consider some of the effects of URT viral infection that are pertinent to their involvement in asthma and speculate on possible mechanisms of virus induced asthma exacerbations.

Bronchial hyperresponsiveness

Increases in bronchial hyperresponsiveness (BHR) have been found in both normal and asthmatic volunteers following infection with rhinovirus^{7,8} and influenza A,⁹⁻¹³ and such increases can be prolonged, lasting, for example, for up to 15 days following infection with rhinovirus.¹⁴ It has also been reported that volunteers with atopic rhinitis who only show an early reaction to allergen challenge can develop both an early and late reaction following experimental rhinovirus infection.¹⁵ These increases in BHR may underlie the development of cough and other lower respiratory tract symptoms in normal individuals, and the increase in severity of asthma that occurs in asthmatic subjects following URT viral infection.

Structural effects on the lower airways

A number of animal models have been used to investigate the effect of URT viruses on lower airways morphology, though work in this area is limited by the few obvious examples of naturally occurring URT viral infections in animals. Two of the early studies described changes due to respiratory syncytial virus infection in the ferret¹⁶ and a poorly adapted strain of influenza in the mouse,¹⁷ both of which gave rise to a cold like illness without an associated pneumonia. Similar changes were seen in both with an early

loss of ciliated and non-ciliated cells from the tracheal epithelium and the basal epithelial layer remaining intact. Regeneration of the epithelial layer began at five days and was complete by two weeks. Studies of chickens infected with viral laryngotracheitis¹⁸ and guinea pigs infected with parainfluenza 3 virus¹⁹ have demonstrated that virus induced epithelial disruption leads to significant increases in epithelial permeability and, as a consequence, an increase in permeability to allergens and an increase in allergen sensitisation.¹⁹ Other workers have studied in some detail the effects of parainfluenza type 1 (Sendai) virus infection in the rat.^{20,21} In this model viral infection also leads to significant morphological changes to the airways with the early development of multifocal necrosis and sloughing of the epithelium lining the bronchi and bronchioles.

Work in humans has also demonstrated an effect of URT viruses on the epithelial integrity of the airways. In one study²² bronchoscopy and biopsy were performed on patients with uncomplicated influenza type A infection. Examination of their airways revealed extensive desquamation of epithelial cells to the level of the basement membrane and thickening, hyalinisation, and distortion of its structure. Other studies have focused on the effects of URT viral infection on ciliary function and have demonstrated delays in tracheobronchial clearance following influenza A infection²³ and depressed nasal ciliary function following experimental rhinovirus infection.²⁴

β adrenergic receptors

An imbalance of β adrenergic receptors has been proposed as a fundamental abnormality in asthma,²⁵ and in vitro studies have demonstrated the ability of viruses to affect β adrenoceptor function. In isolated smooth muscle taken from guinea pigs inoculated with influenza virus the protective effect of the β_2 agonist salbutamol against ovalbumin induced contraction is reduced.²⁶ The nature of this effect has been examined further.²⁷ Mice infected with influenza A that developed infections confined to the URT were compared with those that developed infections of both the upper and lower respiratory tracts. Only in those animals with lower respiratory tract disease was receptor activity reduced with sig-

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nificant decreases in both isoprenaline and NaF stimulated adenylate cyclase activity.

Leucocytes express sympathetic β adrenergic receptors and have proved a useful model of viral/receptor interaction. Granulocytes from asthmatic patients with URT infection show a decreased response to isoprenaline,²⁸ suggesting that decreased β adrenergic receptor sensitivity could contribute to virus induced attacks of asthma. It would not, however, explain the increase in BHR that follows URT viral infection since this phenomenon is not thought to be causally associated with changes in β adrenergic receptor function.

Cholinergic nerves

Cholinergic overactivity could be a mechanism for virus induced increases in BHR since the bronchial hyperresponsiveness that develops following the common cold is blocked by atropine and reversed by isoprenaline.⁷ Evidence from animal models supports this hypothesis and suggests that it is overactivity of the efferent cholinergic nerves that is increased since, in guinea pigs, viral infection has been shown to augment the bronchoconstriction caused by electrical stimulation of the vagus nerve.²⁹ Further work has suggested that viruses may act to increase cholinergic activity by causing dysfunction of the M_2 muscarinic autoreceptor. Treatment with gallamine, an M_2 muscarinic receptor blocker, leads to an increase in airway response to vagal stimulation in uninfected control rats but has much less of an effect in those infected with parainfluenza virus, suggesting that the viral infection has already disrupted the M_2 receptor.³⁰ This disruption would act to increase cholinergic responsiveness since the M_2 receptor is an inhibitory neural autoreceptor, and its blockade would remove the negative feedback loop that limits acetylcholine release from cholinergic nerves.

There are a number of possible mechanisms by which virus infection could cause M_2 receptor dysfunction. Viral neuraminidase has been shown to cleave sialic acid residues from M_2 receptors, decreasing their affinity for agonists, and consistent with this are reports that neuraminidase inhibitors block the effect of parainfluenza 1 virus infection on receptor function.³¹ Another proposed mechanism, which may be more prominent in milder infections, is that receptor dysfunction is caused by inflammatory cell mediated damage.³² It is also possible that mechanisms independent of M_2 receptors may contribute to cholinergic overactivity,³³ and there are a number of candidate substances such as histamine, thromboxane, serotonin, and the tachykinins that can act as parasympathetic neuromodulators³⁴ and could potentially be affected by virus infection. The most important of these, the tachykinins, are discussed below.

Tachykinins

One way in which viral infection could increase cholinergic responsiveness is through modulation of substance P. This tachykinin is con-

tained in unmyelinated sensory airway nerves and can potentiate cholinergic neurotransmission,³⁵ as well as directly cause neurogenic inflammation by inducing extravasation, neutrophil and eosinophil adhesion to vascular endothelium, submucosal gland secretion, cough, and bronchoconstriction.^{36,37} Animal studies have suggested that modulation of substance P catabolism could be a mechanism by which viruses induce exacerbations of asthma. Parainfluenza 1 infection in guinea pigs has been shown to increase substance P induced airway smooth muscle contraction³⁸ and, in the rat, has been shown to amplify substance P induced increases in airway blood flow.³⁹ The effect on smooth muscle seems to be mediated through a decrease in the activity of neutral endopeptidase, an enzyme responsible for the catabolism of substance P.⁴⁰ Rats with a history of viral respiratory tract infection have decreased levels of tracheal neutral endopeptidase when compared with pathogen free rats,⁴¹ and naturally acquired infections of the respiratory tract have been shown to decrease neutral endopeptidase activity in the rat trachea.⁴¹ Furthermore, phosphoramidon, an inhibitor of neutral endopeptidase, potentiates the increase in airway resistance that follows administration of substance P in uninfected guinea pigs but does not do so in guinea pigs infected with Sendai virus, suggesting that neutral endopeptidase is already maximally or near maximally inhibited in virus infected animals.³⁸ The potentiation of airway blood flow induced by viral infection may involve inhibition of angiotensin converting enzyme, which can also modulate substance P activity, as well as inhibition of neutral endopeptidase.³⁹

Cellular and cytokine changes in the airway

Fibreoptic bronchoscopy has allowed examination of the cellular changes in the airway following both experimental rhinoviral infection^{42,43} and naturally acquired colds.⁴⁴ In one study⁴² allergen provocation was undertaken in volunteers with atopic rhinitis before, during, and one month after experimental rhinovirus infection and differences in the early and late phase response were determined by performing bronchoalveolar lavage immediately and 48 hours after each allergen challenge. Important differences were noted in both the early and late phase responses during the acute phase of infection and one month after infection. The early phase reaction was associated with significantly higher levels of histamine in the bronchoalveolar lavage fluid when subjects were challenged during the acute phase of infection and one month after infection. The late phase response was also augmented. When bronchoalveolar lavage fluid at 48 hours following challenge was compared, levels of histamine and, more significantly, numbers of eosinophils were significantly higher when the procedure was performed during the acute phase and one month after rhinovirus infection than when performed before the

infection. These changes did not occur in a control non-atopic group.

In another study⁴³ asthmatic and non-asthmatic volunteers underwent bronchoscopy two weeks before (baseline) and four days (acute) and 6–10 weeks (convalescent) after challenge with human rhinovirus. Bronchial biopsy specimens demonstrated increases in the sub-mucosal CD4+ T cell, CD8+ T cell, and eosinophil counts during the acute phase of infection. CD4+ T cell and CD8+ T cell counts had returned to baseline by convalescence, but eosinophil counts remained raised in the asthmatic group, in contrast to the non-asthmatic subjects whose counts returned to baseline levels. Interpretation of this study is limited since none of the asthmatic volunteers developed asthma symptoms or showed deterioration in spirometric parameters, although there was a significant increase in histamine responsiveness in the asthmatic group. It does, however, show the potential of rhinoviruses to cause airway recruitment of T cells and eosinophils, both important contributors to the inflammatory process, and highlights possible differences in the eosinophilic response between asthmatic and non-asthmatic subjects. It would have been interesting to take this work further by counting the numbers of CD25+ T cells and hence gain an appreciation of the effect of rhinovirus infection on activation of T cells – which is perhaps more relevant than absolute T cell numbers.

A more recent study⁴⁴ has investigated the changes that occur in the lower airways following naturally acquired URT infections. This study found significantly increased numbers of eosinophils and CD8+ T lymphocytes in the bronchial mucosa of a group of atopic and non-atopic subjects following the development of common cold symptoms and a trend towards an increase in CD25+ T lymphocytes. Interestingly, when the changes in the atopic and non-atopic subjects were considered separately, the increase in CD8+ T lymphocytes only reached significance in the non-atopic group. One problem inherent in any study of this type is that the URT infections will be caused by various viruses, each of which may have different effects on the lower airways. In this study 20 subjects underwent bronchoscopy during the acute phase of URT symptoms, eight of whom were atopic and eight (two atopic) had a proven viral infection (two rhinovirus, three coronavirus, one parainfluenza, one respiratory syncytial virus, and one dual infection). Much larger numbers may be required to fully appreciate virus induced changes to the cellular profile of the airways, and certainly subgroup analysis is likely to underestimate any changes.

Virus induced cytokine changes have been studied using the nose as a model. We have studied the cytokine profile of nasal lavage fluid taken during naturally acquired common colds and compared the cytokine response of atopic and non-atopic volunteers. In agreement with previous studies^{45–46} we have demonstrated increased levels of interleukin (IL)-6, IL-8, interferon (IFN)- γ , IL-1 β , and tumour necrosis

factor (TNF)- α in the acute phase of a cold and have shown important differences between the atopic and non-atopic volunteers,⁴⁷ with convalescent levels of IL-6 and IL-1 β remaining significantly higher in the atopic group than in the non-atopic individuals. This is in keeping with the biopsy studies described above in which differences in the response to rhinovirus infection between asthmatic and non-asthmatic subjects only became apparent 6–10 weeks after infection, possibly suggesting that infection promotes a series of inflammatory changes that can be limited in non-asthmatic individuals but not in those with asthma.

Epithelium

URT viruses will first interact with airway epithelium. Epithelial cells express the intercellular adhesion molecule ICAM-1 which is the receptor for the major group of rhinoviruses.^{48,49} Recent work has shown that rhinoviruses have the ability to upregulate surface ICAM-1 expression and cellular mRNA in cultures of pulmonary epithelial cells and can also upregulate surface vascular cell adhesion molecule (VCAM-1) expression.⁵⁰ Such up-regulation could explain an increase in the persistence or severity of inflammation in asthmatic subjects and also the observation that asthmatic children may be more susceptible to colds than non-asthmatic children.⁵¹

The effects of virus infection on epithelial cell cytokine production have been studied in bronchial epithelial cell lines. Viral infection has been shown to promote production of a number of cytokines, as demonstrated by the finding of increased amounts of cytokines in the culture supernatants and increased amounts of mRNA within the epithelial cells (table 1). Upregulation of IL-6 and IL-8 production has been reported following infection of epithelial cell lines with respiratory syncytial virus (RSV)^{52,53} and rhinovirus,⁵⁴ RANTES and macrophage inflammatory protein (MIP)-1 α in response to RSV infection,⁵⁵ granulocyte-macrophage colony stimulating factor (GM-CSF) in response to rhinovirus,⁵⁶ and IL-11 in response to RSV, parainfluenza 3, and rhinovirus.⁵⁷ Rhinovirus induced epithelial cell IL-6 production has been studied in detail using the A549 epithelial cell line and its upregulation is reported to be mediated by an NF- κ B-dependent transcriptional stimulation pathway.⁵⁸

IL-8 is an important neutrophil chemo-attractant and evidence from induced sputum studies suggests that it may have an important role in driving exacerbations of asthma. Induced sputum taken during asthma exacerbations reveals a large group of patients in whom neutrophils are the dominant cell type with increased levels of IL-6 and IL-8 emanating from the lower airways.⁵⁹ IL-8 can also act as a chemoattractant for eosinophils primed by IL-4⁶⁰ and complexed with sIgA. The role of IL-6 is less clear. Receptors for this cytokine are found on activated B cells, T cells, and monocytes and it seems to be involved in T cell activation,⁶¹ in inducing terminal differentiation and immunoglobulin production of

Table 1 Summary of *in vitro* and *in vivo* studies of cytokine production categorised according to cell source and virus type

Cell type	Virus	Experiment	Effect on cell function
Epithelial	Rhinovirus	In vitro	Increased IL-6, IL-8, IL-11, GM-CSF
	RSV		Increased IL-6, IL-8, IL-11, RANTES, MIP-1 α
Peripheral blood mononuclear cells	Rhinovirus	In vivo	Increased IL-6, IL-8, TNF- α
	Measles vaccine	Ex vivo	Increased PHA stimulated IL-2 and IFN- γ
	Influenza A	In vitro	Increased IL-4, TNF- α
CD8+ cells	Lymphocytic choriomeningitis virus	In vitro	Decreased IFN- γ , IL-1 α
		In vivo	Increased IFN- γ and IL-2
Peripheral T cells	Rhinovirus	Ex vivo	Increased IL-4, IL-5 and IL-10
Monocytes/macrophages	Influenza A	In vitro	Decreased T cell response to allergen
Basophils	RSV, adenovirus	In vitro	Increased IFN- α , IFN- β , IL-1 β , IL-6, TNF- α
	influenza, parainfluenza		Increased histamine releasability
	Rhinovirus		Increased histamine releasability
		Ex vivo	Increased LTC ₄ production

RSV = respiratory syncytial virus; IL = interleukin; GM-CSF = granulocyte macrophage colony stimulating factor; IFN = interferon; TNF = tumour necrosis factor; PHA = phytohaemagglutinin; LTC₄ = leukotriene C₄; RANTES and macrophage inflammatory protein (MIP)-1 α are members of the chemokine family.

B lymphocytes, and in regulating pulmonary inflammation and stimulating mucosal IgA immune responses.^{62,63} IL-11 is a pleiotropic cytokine with effects that overlap with IL-6. RANTES is an eosinophil chemoattractant and, consistent with its upregulation by RSV, is the finding that RSV infected epithelial cells induce transendothelial migration of human blood eosinophils.⁶⁴

The finding of virus induced increases in epithelial derived chemokines, such as IL-8 and RANTES, is potentially of great significance since they are directly responsible for the recruitment of inflammatory cells to the airways and there is an increasing awareness of the importance of this group of cytokines in the pathogenesis of allergic inflammation. Much future research is likely to focus on the effects of viruses on the production of other chemokines such as *gro- α* , MIP-1 α , and eotaxin.

Another epithelial derived mediator involved in asthma is nitric oxide (NO).^{65,66} NO may have an antiviral effect⁶⁷⁻⁶⁹ and may be produced as part of the immunological response to URT viruses. It is also implicated as a mediator of increased bronchial blood flow, eosinophilic infiltration, and damage to the airway epithelium,⁷⁰ and may inhibit Th1 T cell proliferation⁷¹ by shifting the T cell cytokine profile towards the Th2 phenotype. Increased levels of exhaled NO have been detected in non-asthmatic volunteers following naturally acquired symptomatic colds with associated lower respiratory tract symptoms⁷² and in asthmatic volunteers following experimental rhinovirus infection. This is in contrast to atopic and non-atopic individuals who develop naturally acquired colds without any lower respiratory tract symptoms, in which no early increase in exhaled NO can be detected.⁷³

T cells

Clinical studies have demonstrated a T cell response following URT viral infection. There is a reduction in circulating T lymphocytes during rhinovirus infection⁷⁴ with increases in

T lymphocyte counts being reported in nasal secretions⁷⁵ and the lower respiratory tract.⁴³

Studies of peripheral blood mononuclear cells have produced conflicting evidence regarding the effect of URT viruses on T cells with reports suggesting that viruses may induce both a Th1 and Th2 type cytokine response. A number of studies have suggested that viruses induce increased Th1 type cytokine production. In one such study⁷⁶ peripheral blood mononuclear cells collected from volunteers five days after experimental rhinovirus infection showed the production of a Th1 type cytokine pattern with significant increases in phytohaemagglutinin (PHA) stimulated IL-2 and IFN- γ production. Consistent with this are reports of increased levels of IFN- γ in nasal lavage fluid during rhinovirus colds.^{47,77} In contrast, others have shown increases in Th2 type cytokine production. Peripheral blood mononuclear cells isolated from healthy volunteers following immunisation with measles vaccine and cultured for 72 hours showed no changes in the proportion of CD4+ T cells, CD8+ T cells, NK cells, or B cells but there was an increased spontaneous release of IL-4 and TNF- α with reduced levels of IFN- γ and IL-1 α .⁷⁸

Although the adoption of a Th1 or Th2 cytokine profile by peripheral blood mononuclear cell cultures suggests activity of CD4+ T cells, there is an increasing realisation that CD8+ T cells, when activated by virus, may make an important contribution to the cytokine pool.⁷⁹ There is evidence for viruses inducing both increases in IFN- γ and release of Th2 type cytokines from CD8+ T cells. Activated CD8+ T cells are major producers of IFN- γ ⁸⁰ and euthymic mice treated with IFN- γ antibodies succumb to an otherwise non-lethal viral infection.⁸¹ Following infection of mice with influenza virus there is a preferential accumulation of CD8+ T cells in the airway and these cells show a high level of IFN- γ mRNA expression.⁸² However, it has also been shown that mouse CD8+ T cells can, in the presence of IL-4, switch to a non-cytolytic CD8-CD4-

Th2 like phenotype producing large amounts of IL-4, IL-5, and IL-10.⁸³ Such a phenotypic switch could be exploited by viruses to reduce the antiviral activity of CD8+ T cells and would also lead to an increase in Th2 type cytokine production. A recent report has suggested that, in the mouse, an ongoing Th2 immune response *in vivo* can switch a virus peptide specific CD8+ T cell to the production of IL-5, leading to airway eosinophilia.⁸⁴ The asthmatic airway, rich in Th2+ T cells, would provide the ideal environment for this switch to occur, and the resulting eosinophilia would lead to a worsening of the asthma.

Thus, studies of virus induced T cell cytokine production present a confusing picture. Both CD4+ and CD8+ T cells may be involved in cytokine production and there is evidence for increased production of both Th1 and Th2 type cytokines. Part of this confusion may be due to the fact that different viral proteins may evoke different responses.⁸⁵ Also, studies have utilised both live virus and vaccines and there are important differences in the way these are processed with live virus being presented, in association with MHC class I proteins, to CD8+ T cells⁸⁶ and dead virus/vaccines presented, in association with MHC class II proteins, to CD4+ T cells. Both responses, however, could be used to explain virus induced exacerbations of asthma. IFN- γ , an important Th1 type cytokine, could contribute to exacerbations by increasing basophil and mast cell histamine releasability (see below), whereas increased production of IL-4 and IL-5 would lead to an amplification of the inflammatory response and an increase in airway eosinophilia.

We have shown interesting changes in circulating T cell function during symptomatic URT infection.⁸⁷ Proliferation of systemic T lymphocytes to house dust mite allergen *Der p* I in sensitised individuals was suppressed during the acute phase of a symptomatic URT infection compared with the convalescent phase, but there were no changes to polyclonal stimulation with the mitogen PHA. The most likely explanation is that allergen-specific T cells are migrating to the airways during the cold, perhaps due to increases in airway permeability to allergen and, since they form only a small part of the total circulating T cell population, the effect of PHA stimulation would remain unchanged. Another explanation is that changes to the cytokine environment may affect T cell proliferation. Production of the Th2 subset of cytokines, either through activation of Th2 cells or through conversion of CD8+ T cells to the Th2 like phenotype, would suppress T cell proliferation.

Monocytes and macrophages

Monocytes and macrophages express high basal levels of intercellular adhesion molecule (ICAM)-1⁸⁸ and act as important constituents of the antiviral response. When infected *in vitro* with influenza A virus, human monocytes display dramatic changes in structure and show signs of activation lasting for 10–12 hours after infection. They mount a potent pro-in-

flammatory cytokine response (table 1) with release of IFN- α , IFN- β , IL-1 β , IL-6, and TNF- α ⁸⁹ which is consistent with the finding of several of these cytokines in nasal lavage fluid. Production of cytokines, with the exception of IFN- β , is further potentiated by the presence of GM-CSF⁹⁰ while stimulation of IFN- α and IFN- γ release is blocked by addition of anti-ICAM-1 antibodies.⁹¹ IFN- α , IFN- β , and TNF- α release occurs in response to live and ultraviolet inactivated virus⁹² whereas release of IL-1 β and IL-6 requires the presence of live virus. The increase in IFN- α is interesting in the context of virus induced exacerbations of asthma since this cytokine may have a protective effect, having recently been shown to inhibit the production of IL-5 by human CD4+ T cells.⁹³ Recent work has also suggested that the protective cytokine, IL-10, may be produced by human blood derived monocytes infected *in vitro* with RSV⁹⁴ and, indeed, IL-10 is expressed in lymph node cells in mice following infection with influenza virus.⁹⁵ IL-10 is an anti-inflammatory cytokine which inhibits the production of cytokines associated with allergic inflammation such as IL-4 and IL-5.⁹⁶ Presumably the protective effect of these cytokines is outweighed by the pro-inflammatory effects of viral infection described above.

Basophil function

Although the role of the basophil in asthma remains controversial, it is known that URT infection leads to major changes in basophil function. *In vitro* incubation of basophils with virus does not in itself cause release of mediators, but a number of viruses (RSV, adenovirus, influenza, and parainfluenza) have been shown to enhance the histamine release that occurs when basophils are stimulated by cross linking of bound IgE with an anti-IgE antibody.^{97,98} These effects have also been shown *ex vivo* with basophils from asthmatic volunteers showing consistently increased levels of anti-IgE stimulated histamine release during the acute phase compared with the convalescent phase of symptomatic colds.⁹⁹ In a recent study we have confirmed these findings and have also shown that URT viral infection augments the increases in histamine release and leukotriene C₄ production that occur in response to cross linking of VLA 4. These effects are specific since there is no increase to the non-specific stimuli of calcium ionophore of F-met peptide.¹⁰⁰

The mechanism of basophil activation is unclear since few basophils will come in contact with viruses in the URT, and systemic spread of URT viruses has not been shown. Activation may involve another mediator released locally by the URT with some studies suggesting that the interferons resulting from the URT may act to enhance histamine release.¹⁰¹

If these observations are relevant one would expect increased levels of histamine in the airways or circulation during symptomatic colds. Studies have suggested increased levels of histamine during URT infection both in nasal

secretions and in plasma,¹⁰² but these have involved children sufficiently ill to be admitted to hospital or subjects developing a late phase reaction following allergen challenge.¹⁵ One study has also shown an increase in histamine levels in nasal secretions of atopic individuals following experimental rhinovirus infection¹⁰³ but, in contrast, a study of 16 volunteers with no history of atopy failed to show any changes in the histamine levels in nasal secretions during naturally acquired colds.¹⁰⁴ We have also failed to show significant increases in nasal secretion histamine levels both in non-atopic and atopic individuals,⁴⁷ although several subjects did appear to have high levels in their lavage fluid. One explanation may be the timing of the acute sample which was taken slightly later (2–4 days) after inoculation in the positive study.

The effect of viruses on the mast cell has not been the subject of much study because of the difficulty of obtaining adequate numbers of mast cells. Animal studies have suggested that similar effects may occur with increased release of histamine from calf mast cells being demonstrated following infection with parainfluenza virus¹⁰⁵ and increased numbers of mast cells have been found in the airway of Brown Norway rats infected with parainfluenza 1 virus.¹⁰⁶

Kinins

Kinins are peptidic hormones formed in tissues and fluids that may be involved in the pathogenesis of asthma through both a bronchoconstrictive and proinflammatory action. Bradykinin, for example, as well as causing bronchoconstriction,¹⁰⁷ increases microvascular leakage in guinea pig airways¹⁰⁸ and increases mucus secretion.¹⁰⁹

Levels of kinins in nasal secretions have been shown to rise following both experimental rhinovirus infection and naturally acquired colds¹⁰⁴ and levels of TAME-esterase activity, which reflects the presence of kinin generating enzymes, have been shown to increase in parallel.¹¹⁰ Some studies¹¹⁰ have shown a positive correlation between levels of kinins and cold symptoms but others have failed to confirm this.¹¹¹

Systemic IgE production

Viral infection has been likened to the onset of atopy¹¹² and increases in systemic IgE levels have been found following infection with the Epstein-Barr virus (EBV),¹¹³ cytomegalovirus (CMV), and the measles virus,¹¹⁴ and following vaccination with whole virion influenza vaccine.¹¹⁵ In one study¹¹⁶ increases in circulating blood IgE were demonstrated in 103 patients with serologically confirmed upper and lower respiratory tract infections. Increases in total serum IgE levels have also been noted following experimental rhinovirus infection.¹¹⁷

Increases in IgE levels could be viral or allergen-specific or could simply represent a generalised upregulation of IgE production. In a small study of 12 children with asthma attacks precipitated by influenza the levels of house

dust mite-specific IgE increased during the acute phase although total serum IgE levels remained unchanged.¹¹⁸ However, other workers have shown a rise in total serum IgE levels following rhinovirus infection with no rise in pre-existing specific IgE levels.¹¹⁷ Others have suggested that the IgE response may be virally directed. RSV infection has been shown to induce the production of virus-specific IgE, the magnitude of the response correlating with degree of wheezing and children with an atopic predisposition developing an IgE response more readily.¹¹⁹ Specific IgE antibodies to parainfluenza virus¹²⁰ and *Mycoplasma pneumoniae*¹²¹ have also been demonstrated.

Although epidemiological studies have shown an association between IgE levels and severity of asthma, there is no evidence that increases in IgE levels are involved in the aetiology of the acute attack. It is more likely that increases in IgE levels are secondary to other changes in the inflammatory pathway. One possibility is that Th1 like T cells are recruited to the site of infection, leading to a predominance of Th2 like cells in the circulation and increases in circulating IgE levels. Another possibility would be the switch of CD8+ cells to the Th2 phenotype resulting in increases in IgE production. A knowledge of the effect of viral infection on local IgE production would be helpful in further developing an hypothesis.

Virus in the lower respiratory tract

The finding of URT viruses in the lower respiratory tract would add potential new mechanisms for the explanation of virus induced exacerbations of asthma. A number of URT viruses – for example, influenza and RSV – can cause pneumonia, providing evidence for their invasion of the lower respiratory tract. Persistent adenovirus has recently been found in the lower respiratory tract of children with a history of wheeze following acute adenovirus bronchiolitis.¹²² Using immunofluorescence adenovirus capsid antigen could be detected in samples of bronchoalveolar lavage fluid in 31 of the 34 children and in all six children in whom culture was attempted its viability was confirmed. In contrast, no virus was found in a group of 20 control children admitted to the same hospital department and studied during the same period.

Evidence that rhinovirus invades the lower respiratory tract is more difficult to obtain. Rhinoviruses grow best at 33°C, the ambient temperature of the URT, thus the higher temperature of the lower airways would be expected to discourage their growth. Temperatures in the tracheobronchial tree lower than 37°C have, however, been described¹²³ and may occur secondary to the mouth breathing that accompanies nasal blockage. Nevertheless, rhinovirus has been recovered from necropsic studies of lung tissue from patients with myelomatosis¹²⁴ and from the lungs of an 11 month old infant dying from asthma.¹²⁵ In addition, viral cultures from sputum are more often positive for rhinovirus than from throat and nasal swabs, suggesting that viral replication may

Figure 1 Summary of the effects of viruses of the upper respiratory tract (URT) that may lead to exacerbations of asthma. IL = interleukin, GM-CSF = granulocyte macrophage colony stimulating factor, RANTES and MIP-1 α are members of the chemokine family, IFN = interferon, TNF = tumour necrosis factor, LTC₄ = leukotriene C₄. URT viruses (centre) have been shown to lead to decreases in β adrenoceptor activity and to cause epithelial disruption leading to exposure of sensory nerve endings, loss of protective mediators, and loss of neutral endopeptidases. They may directly reduce neutral endopeptidase activity and this reduction can lead to increases in levels of substance P and both potentiation of cholinergic responsiveness and the development of neurogenic inflammation. URT viruses have also been shown to act directly on the muscarinic receptor leading to increases in cholinergic responsiveness, and to interact with airway epithelium leading to increases in IL-6, IL-8, IL-11, GM-CSF, and the chemokines MIP-1 α and RANTES leading to B and T cell upregulation and increased numbers of eosinophils and neutrophils. This interaction may also lead to increases in nitric oxide (NO) which may inhibit Th1 proliferation and lead, through increases in levels of IL-4 and IL-5, to an airway eosinophilia and increases in IgE production. URT viruses may act on CD8⁺ T lymphocytes to cause a shift to a Th2 type pattern of cytokine production. They have also been shown to act on monocytes and macrophages leading to increased production of IFN- β , IL-1 β , IL-6, and TNF- α , and to act on basophils leading to increases in basophil histamine releasability (perhaps through increased production of IFN- β and IL-1 β) and LTC₄ production. Finally, URT viral infection may lead to increases in the levels of kinins. All these factors may contribute to the development of exacerbations of asthma following URT infection.

occur in the lower airways,¹²⁶ and bronchoscopic studies following experimentally induced rhinovirus infection have enabled viral isolation from bronchoalveolar lavage fluid.^{127,128}

Necroscopic and bronchoscopic studies suffer from the criticism that the presence of viruses can result from contamination. Conclusive proof relies on the demonstration of viral genome within cells of the lower respiratory tract by *in situ* techniques. These have been developed and used with success to localise rhinovirus in the epithelial cells of nasal mucosa¹²⁹ and must now be applied to the lower airways.

Conclusion

URT viruses are major precipitants of exacerbations of asthma and cause significant increases in BHR in both normal and asthmatic subjects. It is important to understand the mechanisms of virus induced exacerbations of asthma but the paucity of information so far available makes it difficult to propose an all embracing hypothesis.

In this review we have described a number of mechanisms by which URT viral infection may contribute to increases in BHR and the

development of asthma exacerbations. With regard to the immunological changes, the cells and cytokines involved (table 1, fig 1) may differ from those thought to be important in generating the underlying inflammatory response with, for example, CD8⁺ T cells, basophils, and the neutrophil chemoattractant IL-8 assuming a greater importance. Much work needs to be done to delineate further the mechanisms of virus induced exacerbations of asthma, but the understanding of these mechanisms should open up new and potentially useful pathways for therapeutic intervention.

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