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The epidermal growth factor receptor and its ligand family: their potential role in repair and remodelling in asthma

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Key words: asthma; epidermal growth factor (EGF); epidermal growth factor receptor; c-erbB1; epithelial repair.

The bronchial epithelium acts as a physical barrier to the ingress of airborne irritants and plays an active role in the airways immune response, reacting to a range of environmental stimuli to enable interaction with immune and inflammatory cells. It is well established that mucosal damage and epithelial cell shedding are important features of inflammatory airways diseases such as asthma. Evidence now suggests that the repairing epithelium has the potential to contribute to disease chronicity through production of an array of soluble mediators and adhesion molecules. However, the biochemical mechanisms that control epithelial maintenance and repair are poorly understood, even though avoiding or reversing the changes in the airways which are likely to result from chronic inflammation and epithelial restitution remains a significant challenge in asthma therapy. The purpose of this review is to highlight the potential of the epidermal growth factor receptor (EGFR/c-erbB) and its ligands in restitution of the bronchial epithelium. This receptor tyrosine kinase plays a pivotal role in regulation of epithelial cell behaviour, having the capacity to elicit a broad spectrum of cellular responses ranging from migration or proliferation to differentiation and enhanced survival; it also has the ability to regulate expression of various inflammatory mediators, mucins, adhesion molecules, matrix proteins, and metalloproteinases which are relevant to the chronic disease phenotype.

Epithelial injury and activation in asthma

Epithelial damage is a prominent post-mortem feature of fatal asthma. Studies of the airways of patients dying of

Date:

Accepted for publication 18 February 1999

To cite this article:

Davies D.E., Polosa R., Puddicombe S.M., Richter A. & Holgate S.T. The epidermal growth factor receptor and its ligand family: their potential role in repair and remodelling in asthma. *Allergy* 1999, **54**, 771-783.

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ISSN 0105-4538

status asthmaticus (1, 2) have described a marked degree of mucosal oedema, a rich infiltration of the lamina propria by inflammatory cells (particularly eosinophils and neutrophils), and areas of damage to the columnar epithelium, with complete sloughing into the lumen of large sections of epithelium. This is accompanied by an apparent thickening of the basement membrane, dilation of microvessels, smooth-muscle hypertrophy, and increased number and activity of goblet cells and submucosal glands. Detailed cellular and ultrastructural examination of bronchial biopsies and bronchoalveolar lavage fluid have also provided evidence for epithelial damage, even in mild asthma (3–6). These studies suggest that the bronchial epithelium of asthmatics is fragile, with selective loss of columnar epithelial cells from their attachment to basal cells (6). Damage may also be found during disease remission, its extent correlating with bronchial hyperreactivity (BHR) and disease chronicity and severity (7). The causes of airway damage appear to be eosinophil and mast-cell secretory products such as arginine-rich proteins (e.g., major basic protein [8]) and proteolytic enzymes including metalloproteinase 9 (MMP-9, gelatinase B) (9) from the eosinophil, and tryptase and chymase from the mast cell (10). Moreover, environmental stimuli such as allergens may also have direct effects on epithelial integrity through their associated protease activity (11–14). The net result of this damage is a breakdown in the permeability barrier of the bronchial epithelium. This enables tissue-damaging molecules to pass unimpeded from the lumen into the airway wall, thereby providing further stimuli for the progression of mucosal injury.

Damage results not only in structural changes in the asthmatic epithelium, but also in an altered phenotype reflecting increased stress and repair responses. For example, there is an altered profile of adhesion molecule expression including integrins (15, 16), E-cadherin (17), and ICAM (18), as well as local increases in CD44⁺ epithelial cells in areas of morphologic damage (19). Damage also leads to functional changes, with the epithelium becoming a significant source of autacoid mediators, cytokines, chemokines, and growth factors (20–23) with the potential to promote inflammation, fibroblast and smooth-muscle proliferation, and matrix deposition. Many endogenous and exogenous stimuli, including allergens (13, 24), proteases (25), oxidants (26), viruses (27), bacteria (28), and hyperoxia (29), have been shown to activate epithelial cells, probably via specific receptor-mediated processes (e.g., protease-activated receptors [30]) or by induction of a stress response involving reactive oxygen and activation of the proinflammatory

transcription factors NF κ B and AP-1 (31). As this results in expression of a variety of cytokines, including IL-6, eotaxin, IL-8, TNF- α , and GM-CSF, the widespread proinflammatory activity of the bronchial epithelium in asthma might suggest a “stressed” epithelium reacting to a variety of tissue-damaging agents. In asthma, the consequence of this stress response is that the bronchial epithelium is responsible for providing the stimuli that sustain a chronic inflammatory cycle that can perpetuate airways injury (Fig. 1).

Superimposed on this inflammatory cycle is a second cycle directed toward repair of the damaged epithelium (Fig. 1). Although little is known about the processes that drive restitution of the bronchial epithelium, even less is known about whether, or how, the disease process in asthma impairs epithelial repair. A direct link between epithelial damage and airways remodelling has been suggested by studies using guinea pigs (32) in which repeated denudation and restitution of the bronchial epithelium produced reticular basement membrane thickening. Fibroblasts beneath the basement membrane are key cells responsible for the synthesis and secretion of extracellular matrix, and an association between subepithelial myofibroblasts and collagen deposition has been demonstrated in subjects with allergic asthma (33). A recent study has confirmed this finding and has demonstrated that the thickness of the subepithelial collagen is of functional significance, being linked to an increase in bronchial responsiveness and exacerbation of clinical symptoms (34) through alteration of mucosal folding when the airway contracts. A causative link between epithelial damage and airways remodelling has been provided by *in vitro* studies (using polyarginine as a

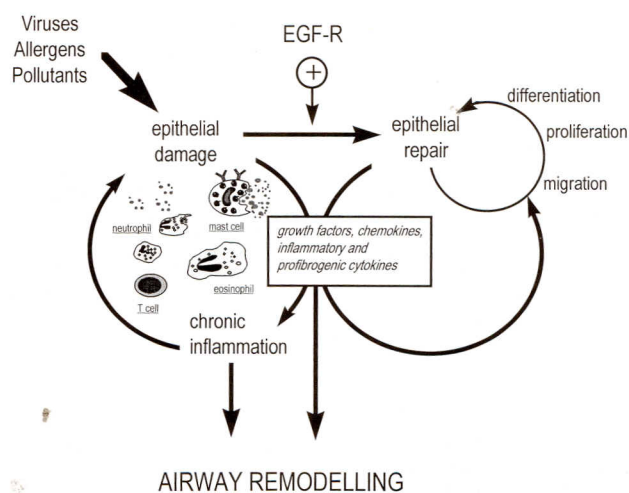


Figure 1. Schematic representation of interactions between epithelial injury, inflammation, and airways remodelling.

surrogate for eosinophil basic proteins) which have shown that chemical or mechanical damage to confluent monolayers of bronchial epithelial cells grown on a collagen gel seeded with human myofibroblasts results in enhanced proliferation of the mesenchymal cells due to the combined effects of bFGF (FGF-2), IGF-1, PDGF-BB, TGF- β , and ET-1 (35). Furthermore, as each growth factor showed a different temporal pattern of release, this might reflect phenotypic changes occurring within the epithelial cells during the process of repair. These data suggest that any defect in epithelial repair could maintain the epithelium in a prolonged state of activation, where, in addition to proinflammatory cytokines, it is responsible for providing proliferative and profibrogenic growth factors that contribute to airways remodelling and disease chronicity.

Bronchial epithelial repair

Initiation of tissue repair occurs as a normal response to epithelial damage. Although the molecular mechanisms which control these processes have not been investigated, it has been shown at the cellular level that the immediate response to damage involves migration of epithelial cells adjacent to the area of damage into the wound to form a temporary squamous barrier (36). The repairing epithelium consists of poorly differentiated and highly spread cells often associated with inflammatory cells. This interim repair is likely to provide polarity and some barrier function, but the cells are unlikely to perform the normal differentiated functions of the epithelium (seromucous secretion, ciliary motility, etc.). Therefore, the epithelial cells in the region of tissue injury must subsequently divide and differentiate to replace the columnar cells that have been lost. This damage-repair cycle in asthma is an ongoing process where more damage can occur at any stage in the cycle, further compromising epithelial integrity and contributing to progressive disease.

Tissue repair is regulated by the activities of inducible genes whose expression is coordinated by intracellular biochemical networks that reflect extracellular signals provided by adhesion molecules and growth factors which convey essential spatial and environmental information to direct responses that are appropriate for a given phase of repair. Among the growth factor receptors, the epidermal growth factor (EGF) receptor occupies a prominent role as a primary regulator of epithelial cell function, having the ability to induce a wide variety of responses, ranging from induction of DNA synthesis, alterations in cell adhesion and

motility, and regulation of differentiated cell function (37). Several lines of evidence suggest important roles for this family of growth factors in cutaneous wound healing (38, 39), and some therapeutic potential has been suggested from their ability to cause a dose-dependent enhancement of epithelialization after topical application (40). Here, we will review the properties of the EGF ligand family and their receptor, the human EGF receptor (EGFR, c-erbB1, HER1), and consider their involvement in lung injury and repair, particularly that occurring in asthma.

The EGF ligand family

Since its discovery in 1962 (41), EGF has been found to have many biological actions. It is best recognized as a potent mitogen for epithelial and mesenchymal cells (reviewed in Ref. 37), but it is also an important effector of specialized cellular function: murine EGF was initially recognized by its ability to accelerate the eruption of teeth and the opening of the eyelids of newborn mice (41), while human EGF (urogastrone) was identified by its ability to cure experimental stomach ulcers in dogs (42) – a property which has subsequently been shown to be due to its ability to inhibit acid secretion from gastric mucosa. Although the interpretation of physiological responses to EGF is hampered by the difficulty in distinguishing between direct and referred response, EGF also has diverse effects on target cells cultured *in vitro*. These effects include stimulation and inhibition of growth, regulation of cellular adhesion and stimulation of cellular motility, enhancement of extracellular matrix production and of matrix-degrading enzymes, stimulation of cellular metabolism, and induction of a transformed phenotype (reviewed in Ref. 37).

EGF is one of six well-characterized ligands for the EGFR (Fig. 2a). The other ligands, which are known to be distinct gene products, are transforming growth factor- α (TGF- α) (43), amphiregulin (AR) (44), heparin-binding EGF-like growth factor (HB-EGF) (45), betacellulin (BTC) (46), and epiregulin (47) (Fig. 2b). Members of the EGF ligand family are characterized by a three-looped structure which is imposed by three conserved intramolecular disulphide bonds (48). In addition to forming a framework for presentation of EGFR-interacting residues, this structural motif may also be responsible for conferring resistance to proteolytic degradation. This may explain how these growth factors can operate in chemically hostile environments such as the gut, the urinary bladder, the lung, and sites of infection or wounding. Despite their structural similarities and the presence of key conserved functional residues, these

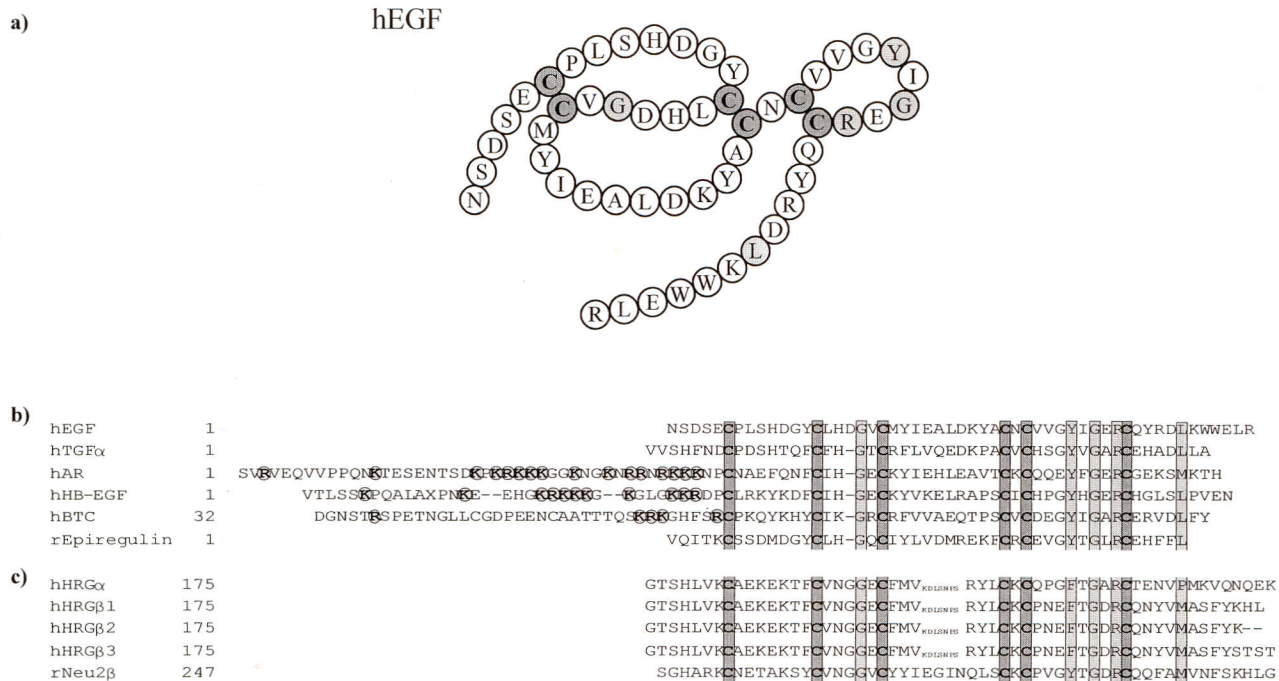


Figure 2. EGF ligand family. a) Secondary structure of human EGF showing three-looped structural motif constrained by three highly conserved intramolecular disulphide bonds between cysteine residues (dark grey). Conserved residues are shaded light grey. b) Clustal W3.1 alignment of sequences for EGF motif of EGFR ligands. Sequences were obtained from SwissProt database. Gaps are inserted into sequences to aid alignment. Extended N-terminal heparin-binding growth factors which contain several basic lysine and arginine residues (grey circles) are also shown. c) Neuregulins show homology to EGFR ligands but exhibit three extra residues within B loop sequence between third and fourth cysteine residues. Amino acids are represented by their single-letter code, and shading of residues is consistent with panel a.

growth factors have markedly different physicochemical properties. For example, they have isoelectric points ranging from 4.6 for EGF (49), 6.8 for TGF- α (43), 7.2–7.8 for HB-EGF (50), and about 7.8 for AR (44). This suggests that their ability to interact with the EGFR, their stability to proteolytic degradation, their intracellular processing, and their capacity to interact with other accessory molecules (e.g., heparan sulphate proteoglycans [HSPGs]) can be markedly different. Indeed, one advantage of this diversity is the ability to function under widely different physiological conditions (e.g., EGF in the gut, HB-EGF at wound sites).

All members of the EGF ligand family are synthesized as transmembrane precursors, with both the membrane-associated and soluble forms exhibiting bioactivity, but with significantly different potencies (51). Thus, the sphere of influence and potency of EGFR ligands can be controlled by juxtacrine, autocrine, and paracrine mechanisms. Cleavage of the transmembrane precursors involves matrix metalloproteinases (MMPs) (52, 53) and can be regulated by protein kinase C-dependent mechanisms or by extracellular calcium influx (54). Cleavage of HB-EGF is regulated by MMP3 (stromelysin-1) (53, 54), whose high-level expression has been reported to occur in asthmatic

bronchial mucosa (55), and which itself is activated by the mast-cell protease tryptase (56).

Comparative studies with EGF, TGF- α , AR, HB-EGF, and BTC show that their physical differences can translate into altered biological potencies. For example, TGF- α is a more potent angiogenic factor than EGF (57), and it is also more potent at releasing calcium from foetal rat long bones (58). One feature of AR and HB-EGF which undoubtedly contributes to their differential ability to activate target cells is possession of a highly basic N-terminal tail which enables them to bind to HSPGs expressed on the cell surface (59, 60). For cells such as keratinocytes which express appropriate HSPGs, this interaction can increase the apparent binding affinity of AR for EGFR by as much as two orders of magnitude, by localizing the growth factor at the cell surface. This localizing effect probably explains the superagonist activity of HB-EGF for vascular smooth-muscle cells (45). Thus, depending on the choice of ligand, receptor activation can be controlled by the ligand's propensity to localize with accessory molecules such as HSPGs or to remain as a diffusible growth factor like EGF or TGF- α . Within a tissue, this could confer a high degree of cellular selectivity; for example, TGF- α released from macrophages may diffuse over a wide area at a wound site,

whereas HB-EGF released from eosinophils or macrophages may be localized to a particular target cell through its expression of a molecule such as CD44v3, whose HSPG component is known to bind HB-EGF preferentially (61). In asthma, CD44 is locally increased in areas of damaged epithelium (19) and may provide an anchor for concentrating the local activity of HB-EGF.

While considerable cellular specificity can be generated by ligand properties which influence the ligand's availability and its ability to interact with the EGFR, cellular responses must also be governed by the phenotype of the target cell. As discussed in the following section, the nature of the signal arising from EGFR activation can be influenced by expression of other members of the c-erbB receptor tyrosine kinase (RTK) family. The presence of these other c-erbB receptors introduces a range of receptor complexes with distinct signalling capabilities.

The c-erbB receptor tyrosine kinase family

The cellular receptor for the EGF ligand family (EGFR, HER1, c-erbB1) is the prototype member of the c-erbB receptor tyrosine kinase family (62). Other members of this family include the structurally homologous receptors c-erbB2 (HER2), c-erbB3 (HER3), and c-erbB4 (HER4) (62, 63) (Fig. 3). Although the heregulin/neuregulin-1 isoforms (HRG/NRG) (Fig. 2c) were initially believed to be ligands for c-erbB2, these and the related HRG2 isoforms have subsequently been demonstrated to bind to c-erbB3 and c-erbB4 (64, 65). A third member of this family, NRG-3, activates EGFR and c-erbB4 (66). At the time of writing, no true ligand for c-erbB2 has been identified.

Within the erbB RTK family, a ligand binding to its cognate receptor results in activation of the intracellular tyrosine kinase and cross-phosphorylation of the receptor dimer; this initiates signal transduction and a biological response by the target cell. The ligand acts by stabilizing erbB receptors in a dimeric form, originally believed to be a homodimer of 1:1 stoichiometry (67). A major advance in understanding c-erbB receptor signalling has come from the recognition that, in addition to stabilizing its cognate receptor as a homodimer, the ligand can also induce its receptor to associate with another c-erbB family member to form a heterodimer (68). These associations appear to follow an hierarchic order of interactions: c-erbB2 appears to be the preferred partner for heterodimerization (69), and its presence is critical for activation of c-erbB receptors by lateral signal transduction (70, 71). The ability of c-erbB receptors to heterodimerize is a particularly important

property because it means that a family member can be activated in the absence of its cognate ligand. Although these interactions were initially described in cancer cells, their importance in normal cells has now been established (72).

Heterodimerization has important functional consequences: treatment of cells expressing various combinations of c-erbB receptors with EGF or HRG- β has been shown to lead to survival, proliferation, transformation, or differentiation (73–76). As each receptor has a kinase with its own substrate specificity (77, 78), can recruit different second messengers (e.g., phosphatidyl inositol 3-phosphate for c-erbB3 (79), and has a different rate of internalization (80) (which is also influenced by ligand properties [81]), differential responses have been attributed to the formation of c-erbB heterodimers and their abilities to activate different intracellular signal transduction pathways. It is likely, therefore, that the pleiotropic effects of the EGFR ligands are mediated, at least in part, by the activity of heterodimeric receptors. This complex network of interactions between c-erbB receptors is further expanded by the finding that BTC (76), HB-EGF (82), and epiregulin (83) exhibit a broader receptor specificity, recognizing c-erbB4 as well as EGFR. While specific ligands also appear to have the ability to interact with selected heterodimeric combinations (84), their significance at physiological ligand concentrations is questionable, as many of these interactions are of low affinity. Overall, there exists a complex combinatorial relationship within the c-erbB receptor and ligand families which offers the potential to regulate finely the molecular and cellular processes that need to be coordinated to effect complex processes such as tissue repair.

Expression of the EGFR and its ligands in human bronchial mucosa

Several immunohistochemical studies have demonstrated expression of the EGFR in human bronchial epithelial tissues, and precise localization of EGFR in adult human lung has been described with immunoelectron microscopy (85). These studies consistently demonstrate EGFR immunoreactivity to be present on the basal cells of the bronchial epithelium. We have recently performed a detailed analysis of the expression of all c-erbB family members in bronchial epithelial cells (86). In these studies, we observed that c-erbB2 and c-erbB3 expression paralleled that of EGFR, suggesting the possibility that these receptors are co-expressed within the same cell and can form heterodimers

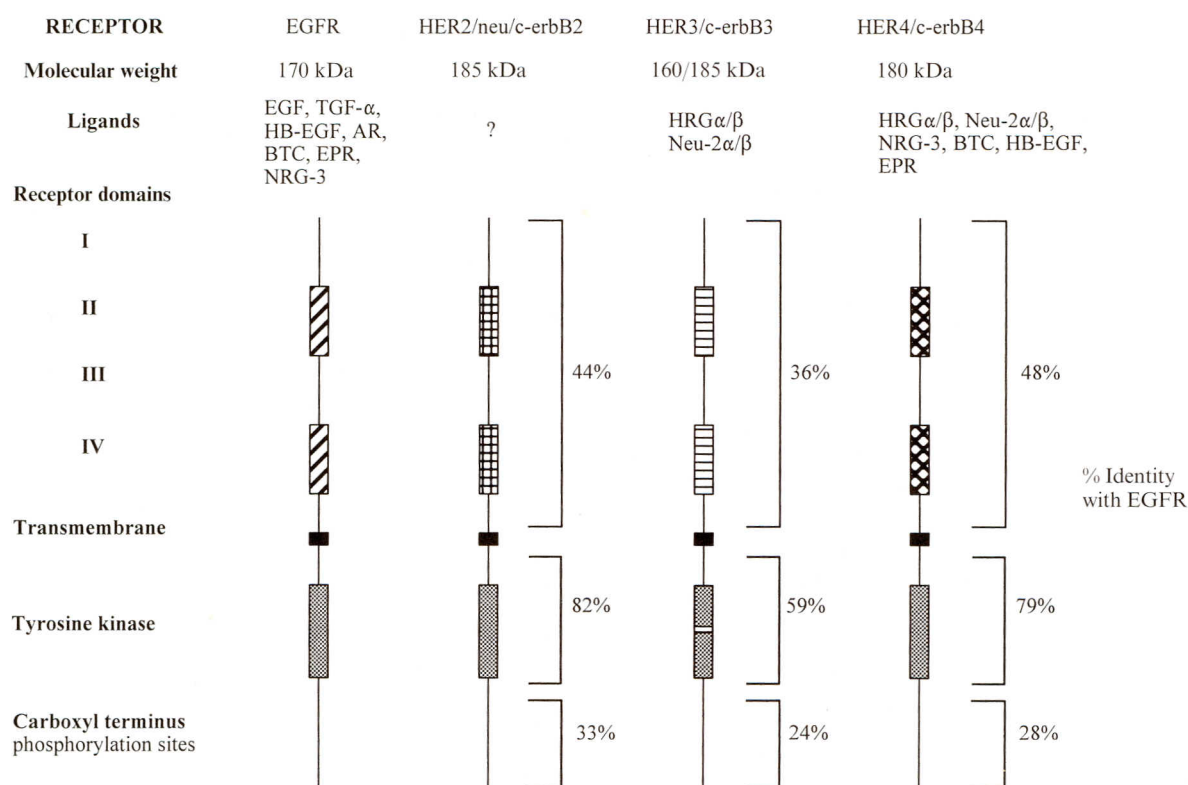


Figure 3. c-erbB family of receptor tyrosine kinases and their respective ligands. Diagram shows sequence identity between domains of human EGFR and human c-erbB2, c-erbB3, and c-erbB4 and ligand specificities of each receptor. Adapted from Ref. 63.

that can regulate intracellular signalling within the basal cells of the bronchial epithelium. Lack of a specific anti-c-erbB4 antibody precluded our determination of the expression of this receptor in the bronchial mucosa; however, our studies using RT-PCR on primary bronchial epithelial cells suggest that c-erbB4 is not expressed by this cell type.

We have also examined the occurrence of EGF, TGF- α , AR, HB-EGF, and BTC in human bronchial epithelium (86). Consistent with a previous immunohistochemical study of EGF expression in human lung [85], we observed that serous acinar cells are a major site of EGF immunoreactivity. It is likely that this is the major source of the EGF-like growth factor which is secreted into the fluid that bathes the apical surface of the bronchial epithelium (87). Furthermore, we also found that BTC was strongly expressed in the serous acinar cells. With the exception of BTC, we also observed immunoreactivity for each ligand within the bronchial epithelium showing a pattern of staining similar to that for the EGFR. As each of these growth factors is synthesized as membrane-bound precursors and is proteolytically cleaved to generate mature growth factors (51–54), detection of immunoreactivity associated with a particular cell need not necessarily identify that cell as the source of the growth factor. However, using RT-PCR, we have shown that bronchial epithelial cells

contain AR, TGF- α , HB-EGF, and EGF mRNA. As bronchial epithelial cells require EGF for growth *in vitro*, it is possible that this is responsible for induction of autocrine ligand expression, as has been observed in cultured keratinocytes (88) and colonic epithelial cells (89).

Although autocrine ligand synthesis was originally proposed to lead to malignancy through uncontrolled stimulation of the EGFR, it is clear that malignant transformation occurs only when the EGFR is expressed at extremely high levels (90). Under conditions of normal receptor expression, autocrine, as well as juxtacrine and paracrine, stimulatory mechanisms for the EGFR contribute to normal cell behaviour. For example, parallel expression of EGFR, EGF, and TGF- α has been observed in developing and postnatal human lung, and it has been suggested that these growth factors regulate lung development and maturation through an autocrine mechanism (91). *In vitro* studies have also demonstrated the importance of an EGFR autocrine loop involving TGF- α and amphiregulin for proliferation of bronchial epithelial cells (92). Furthermore, IFN- γ has been found to induce prostaglandin G/H synthase-2 (PGHS-2) by an indirect mechanism involving upregulation of TGF- α , HB-EGF, and AR, which in turn activate an autocrine EGFR signalling pathway, leading to induction of PGHS-2 expression (93).

The EGFR in lung injury, repair, and remodelling

Several lines of evidence suggest a role for the EGFR and its ligands after acute lung injury and in chronic lung disease such as asthma. Wound repair always includes an inflammatory component, and it is significant that several inflammatory cells found in asthmatic airways release TGF- α and/or HB-EGF. TGF- α is produced by macrophages (94) and eosinophils (95); HB-EGF, which has been purified from wound fluid (96), is produced by eosinophils (97, 98), T cells (99), and macrophages (45); and platelets contain EGF (100). HB-EGF has been identified on the leading edge of keratinocytes in the advancing epithelial margin of regenerating epithelium within partial thickness burn (39). As CD44 is increased in asthmatic epithelium, particularly in areas of damage (19), the CD44v3 variant which can bind HB-EGF (61) may provide a mechanism for selectively targeting HB-EGF to epithelial cells. This association may be of functional significance, for example, in focusing the activity of the EGFR tyrosine kinase on cytoskeletal proteins such as ezrin, radixin, and moesin which associate with CD44 and the actin cytoskeleton (101) (Fig. 4), thereby directing cellular migration into the site of injury.

EGF has been detected in cells of the conducting airways of infants dying of chronic lung disease, but it is not detected in the lungs of infants dying from other causes (102). Although little is known about EGF-mediated regulation of bronchial epithelial repair in man, EGF-like ligands in experimental animals are known to be involved in broncho-alveolar repair through effects on migration (103) and proliferation (104). In a sheep model of smoke-induced tracheal injury, exogenous EGF enhanced epithelial repair (105). Studies of the responses to acute lung injury have shown that EGFR and c-erbB2 expression is induced in type II alveolar cells after endotoxin instillation into rat lung (106). Similarly, expression of EGFR is increased in bronchiolar epithelial cells, alveolar septal cells, and alveolar macrophages after bleomycin-induced lung injury (107) and in bronchiolar cells after exposure to naphthalene (108). These observations are consistent with our own studies of human bronchial epithelium, where we have observed increased immunochemical staining for EGFRs in areas of morphologically damaged epithelium (Fig. 5). These data suggest a role for EGF in the repair of the large airways. The ability of the EGF system to respond to injury in human airways has also been studied in nasal epithelium after exposure to the environmental pollutant ozone, which is known to cause significant epithelial damage. We have

described increased immunostaining for the EGFR, TGF- α , and EGF in the nasal epithelium of healthy individuals after 2-h exposure to ozone at 400 ppb to simulate natural exposure to peak ambient levels (109). These changes are likely to be of considerable functional significance, as perturbation of the level of expression of the EGFR will lead to alterations in the proportions of the various heterodimeric receptor combinations (110), leading to activation of a different subset of signalling intermediates.

In a clinical study, biologically relevant concentrations of TGF- α have been detected in the oedema fluid of patients with acute lung injury (111); moreover, in experimental animals, TGF- α expression increases after bleomycin-induced lung injury (107). TGF- α can upregulate sodium transport and markedly increase net alveolar fluid clearance in rats (112). Furthermore, lung fibroblasts can be induced to secrete TGF- α in response to hyperoxic lung injury (113); in an *in vitro* model, addition of TGF- α markedly accelerated wound closure in mechanically damaged alveolar cells (114) and guinea pig tracheal epithelial cells (115).

Significantly, expression of a TGF- α transgene on a lung epithelial specific promoter resulted in an emphysema-like phenotype with alveoli exhibiting marked enlargement of the airspaces with some interstitial fibrosis (116). In this system, use of an epithelially expressed dominant-negative EGFR demonstrated that the TGF- α mediated its effect via the epithelial cells rather than acting directly on the fibroblasts (117). This highlights the importance of the normal homeostatic mechanisms controlled by epithelial EGFRs in regulating not only the behaviour of lung epithelial cells but also their ability to communicate with the underlying mesenchymal cells. The importance of the EGFR as a regulator of bi-directional communication between epithelial cells and fibroblasts is further illustrated by the phenotype of the EGFR^{-/-} mouse, which has dense, collapsed lungs caused by failure of branching morphogenesis (118). In these mice, the alveoli have features of interstitial lung disease with thickened walls and reduced airspaces. This suggests that any imbalance (either negative or positive) in EGFR signalling can have significant effects on the behaviour of the airways structural cells.

EGFR expression in asthma

It has recently been reported that increased EGFR immunoreactivity occurs in asthmatic bronchial epithelium and in airways smooth-muscle-using tissue (119), supporting the hypothesis that the EGFR is involved in epithelial

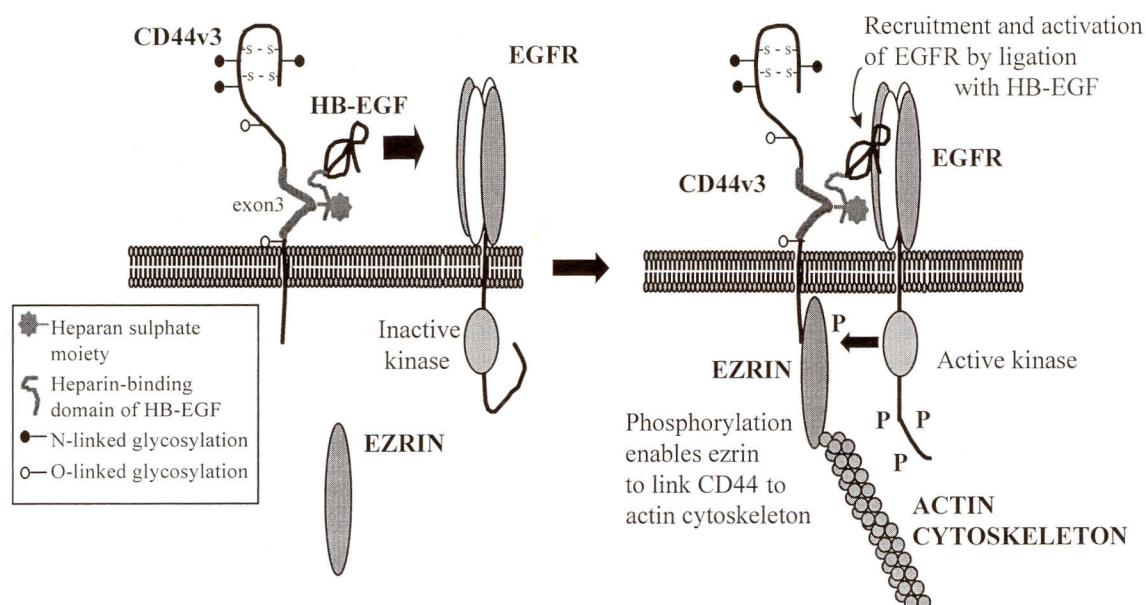


Figure 4. Putative model of interaction between HB-EGF and heparan sulphate moiety of CD44v3, showing local surface regulation of EGFR and actin cytoskeleton through phosphorylation of ezrin.

repair and remodelling. Although this initial study used tissue obtained either post-mortem or from surgical resection for a lung tumour, our own studies of biopsies obtained by fiberoptic bronchoscopy have extended these studies by demonstrating a disease-related increase in EGFR immunoreactivity in asthmatic bronchial epithelium (120). Furthermore, we also observed increased EGFR immunoreactivity in areas of damaged epithelium where the columnar epithelium had been shed to leave a single layer of basal cells. In support of a role for the EGFR in epithelial repair, we have found that either EGF or HB-EGF can stimulate repair of scrape-wounded monolayers of 16HBE 140 bronchial epithelial cells, and that wounding induces tyrosine phosphorylation of the EGFR, even in the absence of exogenous ligand (121). This suggests that EGFR activation is intrinsic to the repair process and may occur by release of an autocrine ligand. Significantly, both mechanical and oxidant damage by hydrogen peroxide results in increased EGFR expression in these monolayers (122). Thus, the elevated levels of EGFR expression seen in morphologically intact areas of the bronchial epithelium of asthmatic subjects may be indicative of widespread functional changes, possibly as a result of microinjury. Alternatively, elevated EGFR expression may reflect the proinflammatory environment in asthmatic lung, as TNF- α is known to enhance EGFR expression in other cell types (123).

Overexpression of the EGFR has been reported in a number of inflammatory diseases such as psoriasis (124), as well as in premalignant and malignant carcinomas (125). In

all of these cases, elevated EGFR expression is associated with hyperplasia driven by an EGFR autocrine loop. Although some goblet-cell hyperplasia is observed, the asthmatic epithelium is not generally considered to be hyperproliferative, as compared with other obstructive airways diseases such as COPD. For example, there is no difference in PCNA expression in asthmatic bronchial epithelium from that of normal controls (126). Therefore, the phenotype of the asthmatic epithelium is not consistent with excessive mitogenic signalling by the EGFR. Indeed, the extent of epithelial damage that occurs in asthma is more suggestive of an epithelium that is either more susceptible to damage or defective in its ability to repair.

Given that the proinflammatory environment of the asthmatic airways is a complex milieu, it is important to recognize that synergistic or antagonistic effects on cell behaviour can arise from altered environmental signals, including those from growth factors, cytokines, extracellular matrix components, or cell adhesion molecules. Indeed, integration of EGFR signalling pathways with other extracellular signals is critical for normal bronchial epithelial cell behaviour. Thus, it has recently been demonstrated that signals from the EGFR, the extracellular matrix, and the retinoic acid receptor are coordinately regulated at the level of p66^{shc} to control mucosecretory differentiation (127). Of the many factors whose expression increases in asthma, TGF- β family members may assume particular importance by virtue of their dual roles as regulators of epithelial and mesenchymal behaviour.

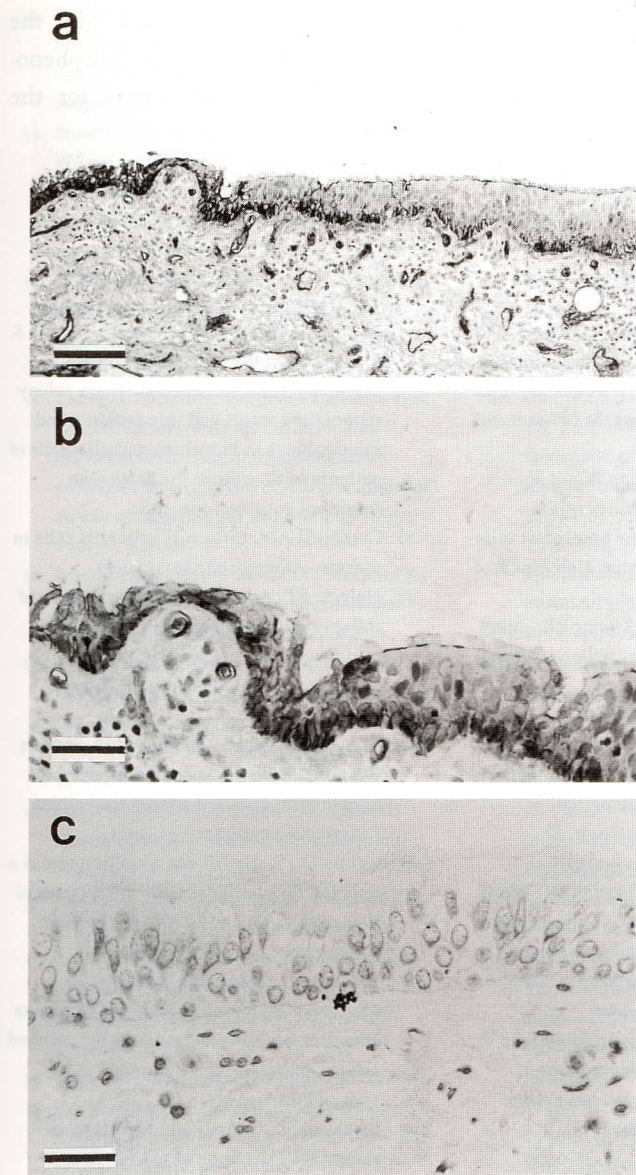


Figure 5. EGFR immunoreactivity in damaged and undamaged areas of human bronchial epithelium. Paraffin sections of bronchial epithelium were stained with sheep anti-EGFR (a and b) or normal sheep IgG (c). In panel a, areas of damaged epithelium show stronger EGFR immunoreactivity than undamaged areas of same sample. Details of intense epithelial immunostaining for EGFR in areas of damage are shown in panel b. c) Negative control. a) Scale bar=100 µm; b, c) scale bars=30 µm.

Increased release of TGF- β has been reported from repairing bronchial epithelial cells, and this may stimulate fibronectin production and migration of epithelial cells (128). However, TGF- β also functions in the later stages of repair, by inhibiting growth and promoting redifferentiation. Growth inhibition of lung epithelial cells by BMP1 (a member of the TGF- β family) has been shown to be due to convergence of signals from the EGFR and the TGF- β receptor at the level of SMAD-1 (129). Hence, proliferative and antiproliferative signals are computed and coordinated

by specific intracellular signalling molecules, so that the balance (or imbalance) determines the normal (or abnormal) response of the cell. Thus, perturbation of the normal balance of mitogenic and antiproliferative factors, as by overproduction or inappropriate expression of TGF- β s, may cause blockade of the proliferative phase of epithelial repair, leading to impaired restitution.

Subepithelial fibrosis and airway smooth-muscle cell hyperplasia are characteristic findings in chronic asthma, and it might be expected that elevated levels of TGF- α , HB-EGF, or EGF are directly involved in stimulating fibroblast proliferation. However, although expression of a TGF- α transgene on a lung epithelial specific promoter results in lung fibrosis (116), use of an epithelially expressed dominant-negative EGFR demonstrated that the TGF- α mediated its effect via the epithelial cells rather than acting directly on the fibroblasts (117). Furthermore, although increased EGF immunoreactivity has been reported in the submucosa of asthmatic subjects (130), the number of cells expressing EGF was not found to correlate with basement membrane thickness or fibroblast number. In contrast, studies of airways smooth-muscle cells have revealed a potentially important role for EGF in smooth-muscle hyperproliferation. The airways contractile agonist, endothelin-1, whose production is elevated in asthma, is not mitogenic for smooth-muscle cells, but it can potentiate the mitogenic effect of EGF (131). Moreover, cysteinyl leukotrienes, which are present in the lung and are released in response to allergen, also potentiate the mitogenic effect of EGF (132). Thus, the effects of EGF on airways smooth-muscle cells may contribute to the pathophysiology of asthma due to its ability to interact with other peptide and lipid mediators.

Concluding comments

In summary, while asthma is an inflammatory disorder, inflammation by itself does not explain many of the features characteristic of this chronic and relapsing disease. In contrast, restructuring of the airway wall as a cardinal feature of the disease may be directly related to disease chronicity and altered airway mechanics. The available evidence points to the disruption and altered functions of the epithelium in asthma as being responsible for the stimuli that lead to restructuring of the airway walls. As a primary regulator of epithelial cell function, the EGFR may assume a prominent role in controlling the bronchial epithelial response to toxic insult, infection, and inflamma-

tion, as well as in the maintenance and repair of this crucial barrier whose function is to protect the airway microenvironment from external stimuli. Further definition of these roles and of the involvement of other c-erbB family members

in these processes should provide new insight into the mechanisms that control the asthmatic epithelial phenotype and may identify novel epithelial targets for the treatment of asthma.

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