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Mechanisms That Potentially Underlie Virus-Induced Exaggerated Inflammatory Responses By Airway Epithelial Cells*

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Mechanisms That Potentially Underlie Virus-Induced Exaggerated Inflammatory Responses By Airway Epithelial Cells*

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Abbreviations: IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; mRNA = messenger RNA; TNF = tumor necrosis factor

Many of the proteins that we tend to measure in asthma as indicators of airway inflammation (*eg*, interleukin [IL]-6, IL-8, granulocyte-macrophage colony-stimulating factor, intercellular adhesion molecule-1, c-jun, and c-fos) or of pathophysiology (*eg*, endothelin-1, β-adrenergic receptor, glucocorticoid receptor, and inducible nitric oxide synthetase) have in common that their encoding messenger RNA (mRNA) is targeted for rapid degradation. This facilitated degradation ensures that mRNA and, thus, protein expression are limited, which is a crucial regulatory mechanism given that most of these labile mRNAs encode for proteins that can initiate and direct, or redirect, responses. Illustrative in this context is that mice in which the proinflammatory mediator IL-6 was overexpressed in the airway epithelium developed major airway pathology.[1] Not surprisingly, therefore, mRNA degradation is strictly regulated, involving complex activation cascades and a range of mRNA-binding proteins.

Our previous studies have focused on the implications of a reduced IL-6 and IL-8 mRNA degradation for IL-6 and IL-8 responses by airway epithelial cells,[2–4] which are key effector cells in the airways. It has been proposed[5] that the degradation of labile mRNAs containing AUUUA sequences in the 3′-untranslated region, as indeed are present in IL-6 and IL-8 mRNA, is dependent on *de novo* protein synthesis. Indeed, the limitation of protein synthesis by synthetic inhibitors resulted in a markedly prolonged half-life for these mRNAs in airway epithelial cells.[2,3] Interestingly, a reduced IL-6 and IL-8 mRNA degradation had profound effects on the dose-response curves (Fig 1).[4] Cells with a reduced IL-6 and IL-8 mRNA degradation showed the following: (1) a steeper dose-response curve, (2) a lowered threshold concentration for a stimulus to induce IL-6 and IL-8, and (3) prolonged IL-6 and IL-8 production. In other words, cells turned from normal responsive into hyperresponsive cells for IL-6 and IL-8 when IL-6 and IL-8 mRNA degradation were reduced. So far, the human airway epithelial-like cell lines NCI-H292 and Calu-3, as well as primary bronchial epithelial cells, displayed this hyperresponsiveness, but neither primary and cell line fibroblasts or peripheral blood mononuclear cells did, which is suggestive of cell-type specificity.[5]

At first sight, a reduced protein synthesis is hard to reconcile with an exaggerated protein (*ie*, IL-6 and IL-8) production. We have verified that this exaggerated production is not due to the release of preformed IL-6 and IL-8, or that it is due to the assays used here to determine IL-6 and IL-8. The current explanation for these exaggerated responses is that, due to a reduced mRNA degradation, IL-6 and IL-8 mRNA steady-state levels increase up to 100-fold, which allows these mRNAs to outcompete other mRNAs for the remaining protein synthesis.

Respiratory viral infections are a major cause of exacerbations in asthma patients.[6] These clinical manifestations are paralleled by the recruitment and activation of inflammatory cells, and have led to studies into the role of proinflammatory mediators during a viral infection. Levels of IL-6 and IL-8 are increased in airway secretions from individuals with a respiratory viral infection. Furthermore, the kinetics and magnitude of IL-6 and IL-8 were found to correlate with respiratory symptoms in influenza infection, thus underlining the prominent role of these mediators in virus-induced pathophysiology. Interferon (IFN)-γ is generated during viral infection and is considered to be a major modulator of innate immune responses.[7] We sought to determine whether and, if so, how IFN-γ modulates IL-6 and IL-8 responses to proinflammatory stimuli (*eg*, tumor necrosis factor [TNF]-α and lipopolysaccharide [LPS]) by epithelial cells. In addition, we assessed whether IL-6 and IL-8 responses are modulated in virus-infected NCI-H292 airway-derived epithelial cells.

NCI-H292 cells that were preexposed to IFN-γ (100 U/mL) for 24 h subsequently displayed exaggerated IL-6 and IL-8 responses to TNF-α and LPS. The underlying mechanism involved the induction of the enzyme indolamine 2,3-dioxygenase, which via depletion of tryptophan reduced protein synthesis and IL-6 and IL-8 mRNA degradation. The addition of exogenous tryptophan largely reversed the reduced mRNA degradation and the exaggerated IL-6 and IL-8 responses.[8]

Parainfluenza virus type 4, which is a member of the pathogenic Paramyxoviruses but in contrast to other members is less cytopathic, was used to assess whether virus-infected cells display a change in the regulation of the IL-6 and IL-8 responses. With time, virus-infected cells displayed a phase with exaggerated IL-6 and IL-8 responses to a secondary stimulus, as exemplified by steeper dose-response curves. This phase also coincided with a

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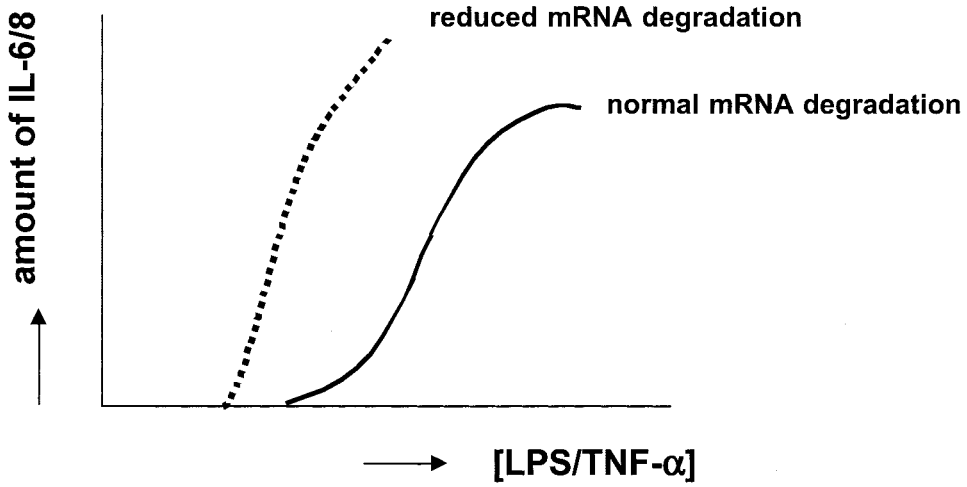

FIGURE 1. Effect of reduced mRNA degradation on the dose-response curves for IL-6 and IL-8.

marked reduction of IL-6 and IL-8 mRNA degradation (Roger et al; unpublished data). Whether this is due to a reduced protein synthesis remains to be shown.

We propose that during a respiratory viral infection, conditions can occur that reduce epithelial IL-6 and IL-8 mRNA degradation, which will lead to exaggerated proinflammatory responses. These *in vitro* findings await confirmation in *in vivo* settings. Whether similar exaggerated responses occur for other proteins encoded by a labile mRNA is unknown.

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REFERENCES

1 DiCosmo BF, Geba GP, Picarella D, et al. Airway epithelial cell expression of interleukin-6 in transgenic mice: uncoupling of airway inflammation and bronchial hyperreactivity J Clin Invest 1994; 94:2028–2035
2 Roger T, Out TA, Jansen HM, et al. Superinduction of interleukin-6 mRNA in lung epithelial H292 cells depends on transiently increased C/EBP activity and durable increased mRNA stability. Biochim Biophys Acta 1998; 1398:275–284
3 Roger T, Out TA, Mukaida N, et al. Enhanced AP-1 and NFkB activities and stability of interleukin-8 (IL-8) transcripts are implicated in IL-8 mRNA superinduction in lung epithelial H292 cells. Biochem J 1998; 330:429–435
4 Lutter R, Loman S, Snoek M, et al. IL-6 protein production by airway epithelial(-like) cells disabled in IL-6 mRNA degradation. Cytokine 2000; 12:1275–1279
5 Sehgal PB, Tamm I. Two mechanisms contribute to the superinduction of poly(I).poly(C)-induced human fibroblast interferon production. Virology 1979; 92:240–244
6 Grunberg K, Sterk PJ. Rhinovirus infections: induction and modulation of airways inflammation in asthma. Clin Exp Allergy 1999; 29(suppl):65–73
7 Billiau A, Heremans H, Vermeire K, et al. Immunomodulatory properties of interferon-gamma: an update. Ann N Y Acad Sci 1998; 856:22–32
8 van Wissen M, Snoek M, Smids B, et al. IFN-gamma amplifies IL-6 and IL-8 responses by airway epithelial-like cells via indoleamine 2,3-dioxygenase. J Immunol 2002; 169: 7039–7044

Actin Dynamics*

A Potential Integrator of Smooth Muscle (Dys-)Function and Contractile Apparatus Gene Expression In Asthma

Parker B. Francis Lecture

Julian Solway, MD; Shashi Bellam, MD; Maria Dowell, MD; Blanca Camoretti-Mercado, PhD; Nickolai Dulin, PhD; Darren Fernandes, PhD; Andrew Halayko PhD; Pawel Kocieniewski, BS; Paul Kogut, MD; Oren Lakser, MD; Hong Wei Liu, MD; Joel McCauley, BS; John McConville, MD; and Richard Mitchell, PhD

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Abbreviations: BHR = bronchial hyperresponsiveness; MAPK = mitogen-activated protein kinase; Raw = airway resistance; SGaw = specific airway conductance; SRF = serum response factor; TLC = total lung capacity

Airway smooth muscle plays a well-accepted and critical role in the pathophysiology of acute airflow obstruction in asthma patients. When stimulated to contract (naturally, by mediators released within the inflammatory environment of the airway wall, or in the laboratory, by the inhalation of methacholine or histamine), the shortening airway muscle bands not only directly narrow the airway lumen, but they also squeeze circumferentially on the submucosa and epithelium, deforming these tissues into folds that invade the already compromised open space for airflow. Together with the excessive mucous secretions

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