Immunity, Inflammation, and Remodeling in the Airway Epithelial Barrier: Epithelial-Viral-Allergic Paradigm

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I. Introduction: Traditional and Alternative Views of Airway Inflammatory Disease

A critical step toward defining molecular mechanisms of airway disease came with formal recognition of the role of immunity and inflammation. In the case of asthma, evidence of immune abnormalities and excessive airway inflammation (induced by allergic and nonallergic stimuli) has led to a widening search for the types of cellular and molecular interactions responsible for linking the initial stimulus to the final abnormality in airway function. It has not yet been possible to integrate all of this information into a single model for the development of airway inflammation and remodeling, but a useful framework has been based on the behavior of the adaptive immune system. In that paradigm, an exaggeration of T-helper type 2 (Th2) over Th1 responses to allergic and nonallergic stimuli leads to airway inflammatory disease, especially asthma. In this review, we summarize alternative evidence that the innate immune system, typified by actions of airway epithelial cells and macrophages, may also be specially programmed for antiviral defense and abnormally programmed in inflammatory disease. Furthermore, this abnormality may be inducible by paramyxoviral infection and, in the proper genetic background, may persist indefinitely. Taken together, we propose a new model that highlights specific interactions between epithelial, viral, and allergic components and so better explains the basis for airway immunity, inflammation, and remodeling in response to viral infection and the development of long-term disease phenotypes typical of asthma and other hypersecretory airway diseases.

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Holtzman, Michael J., Jeffrey D. Morton, Laurie P. Shornick, Jeffrey W. Tyner, Mary P. O’Sullivan, Aurita Antao, Mindy Lo, Mario Castro, and Michael J. Walter. Immunity, Inflammation, and Remodeling in the Airway Epithelial Barrier: Epithelial-Viral-Allergic Paradigm. Physiol Rev 82: 19–46, 2002; 10.1152/physrev.00020.2001.—The concept that airway inflammation leads to airway disease has led to a widening search for the types of cellular and molecular interactions responsible for linking the initial stimulus to the final abnormality in airway function. It has not yet been possible to integrate all of this information into a single model for the development of airway inflammation and remodeling, but a useful framework has been based on the behavior of the adaptive immune system. In that paradigm, an exaggeration of T-helper type 2 (Th2) over Th1 responses to allergic and nonallergic stimuli leads to airway inflammatory disease, especially asthma. In this review, we summarize alternative evidence that the innate immune system, typified by actions of airway epithelial cells and macrophages, may also be specially programmed for antiviral defense and abnormally programmed in inflammatory disease. Furthermore, this abnormality may be inducible by paramyxoviral infection and, in the proper genetic background, may persist indefinitely. Taken together, we propose a new model that highlights specific interactions between epithelial, viral, and allergic components and so better explains the basis for airway immunity, inflammation, and remodeling in response to viral infection and the development of long-term disease phenotypes typical of asthma and other hypersecretory airway diseases.

I. INTRODUCTION: TRADITIONAL AND ALTERNATIVE VIEWS OF AIRWAY INFLAMMATORY DISEASE

A critical step toward defining molecular mechanisms of airway disease came with formal recognition of
the development of airway inflammation include immune
cells as well as parenchymal cells. Cell-cell interactions
are attributed to classes of mediators that include lipids,
proteases, peptides, glycoproteins, and cytokines. The
leading scheme for integrating this information has been
based on the classification of the adaptive immune sys-
tem, and especially the responses of T helper (Th) cells. In
this scheme, CD4\(^+\) T cell-dependent responses are clas-
sified into T helper type 1 (Th1) or type 2 (Th2). Th1 cells
characteristically mediate delayed-type hypersensitivity
reactions and selectively produce interleukin (IL)-2 and
interferon (IFN)-\(\gamma\), whereas Th2 cells promote B cell-
dependent humoral immunity and selectively produce
IL-4, IL-5, and IL-13. Thus Th2 reactions may underlie the
airway hyperreactivity and inflammation characteristic of
the late response to allergen inhalation and so account for
the overproduction of Th2-derived cytokines that is char-
acteristic of asthma (109, 151). How then can a Th2-
polarized response account for asthma that is also trig-
gerated by exposure to nonallergic stimuli (especially
respiratory viruses) that would ordinarily trigger a Th1
response? On the basis of the possibility that Th2-skewed
responses may develop in this setting as well (21, 62, 121),
it is possible that a Th2-dominant response may mediate
inflammation and hyperreactivity in response to nonaller-
gic stimuli as well (as modeled in Fig. 1). However, this
dichotomous view of the immune response may not be
completely accurate, since there is often ambiguity in the
type of Th response triggered by most stimuli as well as
significant cross-regulation between the two types of re-
sponses (57). In fact, recent studies in mice using adap-
tive transfer with Th1 and Th2 cells have indicated that
Th1 cells may even be involved in initiating the allergic
response (16, 110).

Because of these uncertainties, we have questioned
whether other aspects of immunity and inflammation
might also be critical for the pathogenesis of airway dis-
ease. In particular, we aimed to develop a model that
better accounted for the dissociation between the develop-
ment of allergy and asthma in many subjects and was
based on a more precise appraisal of the Th1 system in
the airway. Furthermore, the “Th2 hypothesis” does not
account for the possibility that the airway epithelial cells
may act as active sentinels of innate immunity, and so,
like other components of the innate immune system, may
provide critical signals to the adaptive immune system.
This review summarizes how our view of the barrier
epithelial cell has evolved based on the identification of
its specialized programming for host defense and abnor-
mal programming in disease.

The review is divided into six major sections. Section
\(\text{II}\) summarizes studies of isolated airway epithelial cells.
The section proceeds from a relatively simple scheme for
leukocyte recruitment (based on cell adhesion and che-
moattraction) to one that depends on the coordinated
expression of a network of epithelial immune-response
genes under distinct transcriptional and posttranscrip-
tional controls. The transcriptional program depends prin-
arily on interferon-Jak/Stat signaling while posttranscrip-
tional regulation uses a distinct RNA-protein
interaction that is responsive directly to viral replication.
Section \(\text{III}\) summarizes studies that extend these same
molecular pathways to studies done in vivo using a mouse
model of viral bronchiolitis that takes advantage of a
generically defined host. Section \(\text{IV}\) summarizes studies of
human subjects with airway disease, focusing on how
these same epithelial gene networks behave in subjects
with stable or flared asthma in the presence or absence of anti-inflammatory treatment with glucocorticoids. This section presents a model for how the epithelial gene network might interact with the Th1/Th2 balance to cause airway inflammation in asthma and introduces the possibility that this same gene network may also underlie persistent abnormalities in airway epithelial mucosal behavior found in chronic airway disease. Section V summarizes studies of this possibility done in vitro and in vivo in mice and humans, highlighting the role of genetic susceptibility to virus-induced modification of epithelial phenotype and consequent remodeling. Section VI, the final section, summarizes the review.

II. MOLECULAR BASIS OF AIRWAY EPITHELIAL IMMUNE FUNCTION FROM IN VITRO STUDIES

This section summarizes studies of isolated airway epithelial cells, placing special emphasis on the use of primary-culture human cells that exhibit high-level fidelity to in vivo behavior. Section II A begins with a model of epithelial barrier function and so presents a working scheme for epithelial-dependent traffic of immune cells into the airway. This model depends on at least one major ligand for cell adhesion, i.e., intracellular adhesion molecule-1 (ICAM-1) and another for chemotaxis, i.e., regulated upon activation, normal T cell expressed and secreted (RANTES) chemokine, each of which depends on the level of gene expression for function. Accordingly, section II C and D, proceeds to summarize the basis for epithelial expression of these two corresponding genes. In doing so, these studies define two larger gene networks. One is typified by ICAM-1 and is under the control of IFN-driven Jak/Stat signal transduction and uses Stat1 as a key intermediate. The other is typified by RANTES and relies on posttranscriptional regulation that is more directly inducible by viral replication. In this section, we also introduce recent findings related to the IL-12 p40 gene product that also appears chemotactic, but the regulation of this pathway is not yet fully defined. Later sections will summarize evidence for the role of these same systems in mediating immunity and inflammation in vivo in animal models and human subjects.

A. Gene Products for Mediating Transepithelial Traffic of Immune Cells

The initial approach to defining epithelial control of immune cells and specific epithelial-leukocyte interactions relied on studies of immune cell interactions with each other and with the endothelium. In these systems, the development of monoclonal antibodies against immune cell determinants indicated that a critical step in leukocyte influx from the circulation is the coordinated interaction of specific cellular receptors on the migrating leukocytes with corresponding ligands on adjacent endothelial cells. A scheme for leukocyte recruitment was developed that revolved around the regulated expression of distinct families of cell communication molecules, notably the selectins, the integrins, and the cell adhesion molecule (CAM) members of the immunoglobulin (Ig) supergene family (12). In addition to these cell adhesion systems for direct cell-cell contact, endothelial cells and immune cells also appear capable of generating a series of chemoattractants [e.g., platelet activating factor (PAF)] and chemoattractant cytokines (or chemokines) that may act over a greater distance to direct immune cell movement and activation in tissue (85). These three systems, 1) selectin binding to carbohydrate mucinlike molecules, 2) cell adhesion molecules from the Ig and the extracellular matrix protein families binding to integrins, and 3) chemoattractants and chemokines binding to G protein-coupled receptors, may act in a specific combination to dictate the type of immune cells that enter and get retained in the tissue. Especially in the case of endothelial-leukocyte interaction, this information has enabled the development of a stepwise molecular scheme for leukocyte influx into tissue from the circulation (120).

1. Cell adhesion molecules and the role of ICAM-1 in the epithelium

Our initial studies of epithelial-immune cell adhesion were undertaken with airway epithelial cell monolayers established under conventional cell culture conditions, thereby exposing only the apical epithelial surface to the immune cell (an approach that is more logically taken for studies of endothelial cells). Improving on earlier methods, however, we studied the basis for immune cell adhesion to airway epithelial and venular endothelial cells using a quantitative flow cytometry-based assay (98). This technique avoids extensive leukocyte purification, culture, or labeling steps that may alter leukocyte function and by their nature may eliminate heterotypic cell-cell interactions that may be important in vivo. The quantitative aspect of the flow cytometry methodology was also critical for examining the low levels of constitutive adherence that might be expected for resting T cells. Compared with standard cell-labeling methods, the flow cytometry-based assay yielded a lower level of constitutive T cell adhesion despite a similar level of stimulated adhesion (after T cell activation with phorbol dibutyrate) using endothelial or epithelial cell monolayers. Endothelial-T cell adhesion was further increased by monolayer treatment with tumor necrosis factor-α (TNF-α) (less so with IL-1β and least with IFN-γ), whereas epithelial-T cell adhesion was most sensitive to IFN-γ. Cytokine stimulation of adhesion was invariably concentration dependent and...
closely matched to the cellular levels of ICAM-1 (79). Accordingly, T cell adhesion was markedly inhibited by anti-ICAM-1 or anti-β2-integrin antibody (95–97% inhibition for endothelial cells and 57–67% inhibition for endothelial cells) directed against ICAM-1 interaction with leukocyte function-associated antigen 1 (LFA-1). Residual endothelial-T cell adhesion that correlated with endothelial vascular cell adhesion molecule-1 (VCAM-1) levels was blocked by an anti-α4-integrin antibody directed against VCAM-1 interaction with very late-activation antigen 4 (VLA-4). The results suggest that 1) peripheral blood T cells without exogenous activation exhibit little LFA-1- or VLA-4-dependent adherence except to endothelial or epithelial cells expressing high levels of ICAM-1 and/or VCAM-1; and 2) differences in endothelial versus epithelial cell mechanisms to bind activated and unactivated T cells (e.g., dependence on a mixed- versus a single-ligand system and distinct cytokine-responsiveness of ligand levels) may help to coordinate T cell traffic to epithelial barriers. These findings also support the view that T cell trafficking into inflamed tissues (especially in mucosal barriers) depends critically on local activation events.

These initial studies highlight two distinct features of airway epithelial cell behavior: relative hypersensitivity to IFN-γ and a critical role for ICAM-1 in epithelial-immune cell adhesion. This pattern of cell adhesion molecule expression and function on airway epithelial cells appears distinct from the one for venular endothelial cells. In the case of endothelial cells, at least three interactions of cell adhesion receptors have been implicated in the development of inflammation in epithelial barriers: 1) E-selectin binding to its leukocyte sialyl-Lewis X carbohydrate ligand (38, 49, 145); 2) VCAM-1 binding to the leukocyte β2-integrin VLA-4 (α4β1) (7, 97); and 3) ICAM-1 binding to the leukocyte β2-integrins LFA-1 (αLβ2) or Mac-1 (αMβ2) (101, 146). In each case, there is evidence that the ligand may be overexpressed and/or activated under basal or allergen-challenge conditions in asthmatic subjects and that pretreatment with a blocking monoclonal antibodies can inhibit immune cell influx in a model of asthmatic airway inflammation. The findings provide for two systems (E-selectin and VCAM-1) capable of initiating leukocyte rolling and tethering to the vascular endothelium as well as two that may mediate diapedesis through the vascular wall (VCAM-1 and ICAM-1). However, neither isolated cells in culture nor endobronchial tissue from normal and asthmatic subjects indicated that airway epithelial cells (or fibroblasts or smooth muscle cells) expressed significant levels of E-selectin or VCAM-1. These findings are therefore consistent with the view that airway epithelial cells present fewer adhesion ligands (than endothelial cells) for directing leukocyte movement. The biologic basis for this difference may reflect the capacity of endothelial cells to be armed with ligands that slow down passing leukocytes (to allow tethering and triggering) and that select a specific leukocyte subset (to allow adhesion and transmigration) from the diverse circulating pool. This requires multiple molecular interactions with varying specificities. In contrast, other parenchymal cells may come into contact with immune cells after some degree of selection and activation so that they are required only to facilitate further leukocyte migration and retention. ICAM-1 is well suited to mediate this process because nearly all immune cells constitutively express its receptors, i.e., the β2-integrins LFA-1 and Mac-1. In the case of T cell traffic, the endothelial cells (and likely epithelial cells) also may express a series of receptors (designated homing receptors or addressins) that serve to direct distinct subsets of lymphocytes to appropriate locations of lymphoid tissue. This type of cell adhesion (exemplified by some of the β2-integrin interactions) is probably most important in maintaining a resident population of immune cells, but whether this system is also regulated during airway inflammation remains uncertain.

Interestingly, a specific addressin for T cell localization to the lung has not yet been identified. We reasoned that patterned expression of more common adhesion molecules might also facilitate traffic of specific T cell subsets to the airway mucosa. To define the relationship between T cell phenotype and adhesiveness, we examined T cell adhesion to endothelial cell, fibroblast, and epithelial cell monolayers and extracellular matrix proteins (collagen and fibronectin) using a three-color flow-cytometry-based adherence assay that minimizes basal adhesion levels and facilitates quantitative lymphocyte subtyping (99). Regardless of monolayer type, monolayer stimulation conditions, or T cell activation status, we found that the γδ-TCR bearing T cells adhered more efficiently than αβ T cells. The difference was based predominantly on increased levels of activatable LFA-1 (and to a lesser degree VLA-4) because 1) it correlated precisely with inhibitory function by anti-LFA-1 (and VLA-4) monoclonal antibodies and the levels of LFA-1 (and VLA-4) on the cell surface; and 2) it persisted after maximal LFA-1 (and VLA-4) activation with phorbol dibutyrate. In contrast to most cases of αβ T cell behavior, γδ T cell adhesion to cell monolayers was not linked to memory status, i.e., there was no difference between naive Vδ1+ and memory Vδ2+ populations in levels of LFA-1 (or VLA-4) expression or LFA-1(- or VLA-4-)dependent adhesion to cell monolayers. However, Vδ1+ cells exhibited higher levels of VLA-5 that correlated with an increased adhesiveness to fibronectin and to a 120-kDa fibronectin fragment (FN-120) that contains only the VLA-5-binding domain but not to type I collagen or to a fibronectin fragment (FN-40) that binds only VLA-4. Taken together, the results define a hierarchy for integrin (LFA-1, VLA-4, and VLA-5) expression and consequent adhesion among T cell subsets that is linked to TCR gene usage (but not necessarily linked to memory...
status) and may thereby help to explain the accumulation and retention of V61+ γδ T cells in epithelial and connective tissues. The findings underscore the general concept that immune cells (like parenchymal cells) may regulate their expression of cell adhesion molecules to achieve preferential localization in tissues. The relationship of regulation of more general cell adhesion receptors (such as LFA-1) to more specific systems directed by addressins or by specific antigens needs to be further defined. At present, however, it appears that these more general receptors may work in concert with more specific ones, a concept that is illustrated during antigen presentation where cell adhesion molecules facilitate engagement and activation of the T cell receptor for antigen in the appropriate major histocompatibility complex (MHC) context. Recruitment of specific subsets of leukocytes may also be mediated by a diverse capacity for epithelial production of chemokines as noted in section 11A2.

2. Chemokines and the role of RANTES in the epithelium

As noted above, schemes for transendothelial movement of immune cells (extravasation) depend on the coordinated expression of cell adhesion molecules and chemotaxins that interact with corresponding receptors on the immune cell surface (12, 85, 120). Thus it appeared reasonable to propose that similar molecular mechanisms may regulate transepithelial movement of immune cells. However, there are critical differences between epithelial and endothelial cell adhesion and transmigration, the most obvious of which may be that immune cell recruitment through endothelium and epithelium generally occur in opposite directions with respect to the luminal surface of cells. For endothelium, immune cells leave the circulation in an abluminal direction moving from apical-to-basal endothelial surfaces, whereas for epithelium, cells move toward the lumen traversing a basal-to-apical direction with respect to epithelial cell surfaces. In the case of the endothelium, this directional process may be coordinated by the actions of selectins, cell adhesion molecules, and chemokines all acting as haptotaxins (90), but the driving force for immune cell movement across the epithelial barrier (if it exists at all) was uncertain. Thus expression of epithelial ICAM-1 and consequent interaction with T cell LFA-1 appeared to be the major determinant of T cell adhesion to the apical surface of the airway epithelial cell monolayers (98, 99), but the extent to which ICAM-1/LFA-1 interaction or other molecular interactions might regulate T cell traffic through the epithelium and the directionality of movement was uncertain for airway and other epithelia.

Accordingly, we next developed a system for monitoring immune cell adhesion and transmigration through an epithelial model in apical-to-basal and basal-to-apical directions (120). Immune cell (in this case, T cell) behavior was again monitored by quantitative flow cytometry to avoid a need for extensive leukocyte purification, culture, and labeling. Epithelial monolayers were established with primary-culture human tracheobronchial epithelial (hTBE) cells that exhibit differentiated structural and functional features of polarized epithelial barriers found in situ (149). In particular, monolayers of hTBE cells emulate in vivo behavior with low basal levels of ICAM-1 and cytokine-dependent increases in ICAM-1 expression (66, 79, 114). In this ex vivo system, T cell adhesion and subsequent transmigration were blocked in both directions by monoclonal antibodies against LFA-1 or ICAM-1 (induced by IFN-γ treatment of epithelial cells). The total number of adherent plus transmigrated T cells was also similar in both directions, and this pattern fit with uniform presentation of ICAM-1 along the apical and basolateral cell surfaces. However, the relative number of transmigrated to adherent T cells (i.e., the efficiency of transmigration) was increased in the basal-to-apical relative to the apical-to-basal direction, so an additional mechanism was needed to mediate directional movement toward the apical surface. Screening for epithelial-derived T cell chemokines indicated that IFN-γ treatment caused predominant expression of RANTES (68). The functional significance of RANTES production was then demonstrated by inhibition of epithelial-T cell adhesion and transepithelial migration by anti-RANTES monoclonal antibody. In addition, we found that epithelial (but not endothelial) cells preferentially secreted RANTES through the apical cell surface, thereby establishing a chemical gradient for chemotaxis across the epithelium to a site where they may be retained by high levels of RANTES and apical ICAM-1. In this system, RANTES did not account for all chemotactic activity, and subsequent studies have indicated that other chemokines (and other chemotaxins such as IL-12 p80) provide for functional redundancy (see sect. III). Nonetheless, the results define how the patterns for cell-specific apical sorting of RANTES serve to mediate the level and direction of T cell traffic and provide a basis for how this process is precisely coordinated to route immune cells to the mucosal surface and maintain them there (Fig. 2). Potent effects of RANTES on T cells (4), eosinophils (2), and macrophages (as described below), distinct from effects on cell movement, indicate an additional role of epithelial cells in regulating the activation status of local immune cells.

In sum, it appears that airway epithelial-T cell adhesion and transmigration depend on the cytokine-dependent expression of ICAM-1 on the epithelial cell surface (98), concomitant levels of expression and activation of LFA-1 on the T cell surface (99), and polarized secretion of RANTES through the apical cell surface (120). For ICAM-1, distribution on both cell surfaces mediates efficient cell adhesion at the basal cell surface (to aid in transmigration) and at the apical cell surface (for retention and movement along the airway). For RANTES, the pattern of preferential apical secretion provides for a soluble chemical gradient for T cell movement from the subepithelium (where levels are low) to the mucosal surface (where levels are higher). These patterns fit well with available data for
ICAM-1 and RANTES expression in airway epithelium in vivo (see below), but we emphasize that this pattern of cell adhesion molecule expression and chemokine secretions is distinct. Other types of epithelial cells (notably colonocytes and pneumocytes) express ICAM-1 only along the apical cell surface (11, 59, 106). Perhaps in this setting, ICAM-1 functions mostly in cell movement along that surface or acts in concert with other cell adhesion receptors to aid in host defense (e.g., as opsonins) (102). Similarly, epithelial capacity for polarized secretion of RANTES may be distinct from other cell types. In the case of endothelial cells, it appears that chemokines with immobilization domains (including RANTES and IL-8) are anchored to the cell surface to act as haptotaxisins rather than freely secreted to act as chemotaxisins (41, 113, 131). Apparently in this haptotactic mode, there is no mechanism for polarization because both luminal and abluminal surfaces of endothelial cells are coated with chemokine (90). It is also possible that the cellular source of endothelial-bound chemokine is the epithelial cell, since transcytosis and subsequent presentation of exogenous chemokine may occur in either direction. Taken together with comparative data for endothelial cells, our studies of airway epithelial cell-T cell interaction offer a means for progressive movement of T cells from endothelium to the airway lumen through distinct cell-specific mechanisms for cell adhesion molecule expression and chemokine secretion. As noted below in studies of human subjects, these same molecular systems appear activated in asthma because there are increased numbers of activated T cells as well as increased levels of ICAM-1 and RANTES expression in the epithelium of asthmatic subjects depending on the severity of disease and treatment conditions.

B. Cytokine-Dependent Gene Network: Transcriptional Regulation

Section II concentrated on the topological basis for how epithelial cells coordinate their expression of cell...
adhesion molecules and chemoattractants with that in the underlying vascular plexus so that leukocytes migrate efficiently through the tissue. Another challenging question is how this process is coordinated to be stimulus specific. The first clue to this process was the finding that vascular endothelial and airway epithelial cells have distinct but complimentary profiles of cytokine responsiveness for induction of ICAM-1 and ICAM-1-dependent cell adhesion and transmigration. In particular, it appears that airway epithelial cells exhibit a selective sensitivity to IFN-γ (79), and this selectivity in cytokine responsiveness of ICAM-1 expression offered an opportunity to decipher the genetic code of airway inflammation. In general, inducible gene expression may be mediated by signal transduction leading to regulation at transcriptional or post-transcriptional levels (controlled by DNA- or RNA-protein interactions, respectively). In the case of the ICAM-1 gene, nuclear run-off assays aimed at monitoring transcriptional initiation rate indicated that IFN-γ regulation of epithelial ICAM-1 levels occurs at least in part at a transcriptional level (80). Because gene transcription is often regulated by DNA-protein interactions in the promoter region, the basis for selective cytokine control of ICAM-1 expression could be investigated by structure-function analysis of the ICAM-1 gene promoter region. This section therefore summarizes findings on one aspect of this genetic code: the DNA-protein interactions in the ICAM-1 gene promoter region that control IFN-γ-inducible transcription of the ICAM-1 gene. As will be developed, the molecular building blocks used to regulate the ICAM-1 gene are also used to control an entire immune-response gene network in the epithelium.

Initial identification of DNA-protein interactions is often undertaken with a functional analysis of promoter/reporter gene constructs and a concomitant analysis of proteins that bind to any identified regulatory sites. When this approach was used to analyze IFN-γ-inducible expression of the ICAM-1 gene in primary-culture airway epithelial cells, a 37-bp region of the gene from nucleotides −130 to −93 was found to be responsible for selective IFN-γ responsiveness of airway epithelial cell ICAM-1 expression (80). This region (designated the IFN-γ-response element or IRE) contained an 11-bp inverted repeat (the gamma-activation site) that was necessary and sufficient for IFN-γ-responsiveness of the ICAM-1 gene. This function was closely linked to the capacity of this site to bind to Stat1 (the first member of the signal transduction and activation of transcription factor family). Because vascular endothelial cells also appear capable of Stat1 activation and binding in response to IFN-γ treatment, their failure to fully respond to IFN-γ may be due to the presence of a concomitant interaction of a repressor protein that prevents the response. This possibility may be reflected by restoration of IFN-γ responsiveness in endothelial cells by protein synthesis inhibition with cycloheximide treatment (D. C. Look and M. J. Holtzman, unpublished observations). The relatively small ICAM-1 response to IFN-γ in endothelial cells may be reconciled with the fact that IFN-γ-targeted viruses are not often encountered at the endothelial barrier. However, as discussed next, the shared capacity of both endothelial and epithelial cells to utilize an alternative DNA element for IFN-γ-activation of the MHC class I genes may provide a common pathway for antigen recognition and processing that is essential for immune defense against inhaled as well as circulating agents.

Additional studies of airway epithelial cells in the context of work on other cell types by us and others indicate that transcriptional regulation of the epithelial ICAM-1 gene is typical of genes controlled by an IFN-γ-responsive Janus family kinase (Jak)-Stat signaling pathway (summarized in Fig. 3). This pathway consists of the IFN-γ-receptor, receptor-associated Jak1 and Jak2 tyrosine kinases, the Stat1 transcription factor, and specific Stat1/DNA and Stat1/protein interactions in the ICAM-1 gene promoter region (79, 80, 141). Signaling depends on the capacity of Stat1 to relay the signal from the cytoplasm to the nucleus through its capacity to interact both with the IFN-γ-receptor α-chain (46) as well as the GAS site in the gene promoter region (80). In addition to Stat1/DNA interaction, it appears that Stat1 interaction with the constitutively active transcription factor Sp1 is also critical for full activation of the ICAM-1 gene (81). At the promoter, direct and indirect interactions with the coactivators p300/CREB and p/CIP may further facilitate enhanceosome formation and more efficient gene transcription, and these components appear active in airway epithelial cells as well (58, 82, 134, 153). In other cell systems, an additional action of a serine/threonine mitogen-activated protein kinase is needed to convey full activity, but this step appears less influential in airway epithelial cells (141).

In further work, it appears that these same molecular building blocks serve to regulate IFN-γ responsiveness of other epithelial immune-response genes. Thus Stat1 binding also confers IFN-γ responsiveness of genes for transporter and antigen processor-1 (TAP-1), interferon regulatory factor-1 (IRF-1), and Stat1 itself (80–82, 115, 141). As discussed further below, induction of Stat1 and consequent autoamplification of this pathway may serve to exaggerate the inflammatory response in experimental models and in disease. Similarly, the IRF-1 gene product is also a member of a transcription factor family and so may amplify the pathway (in concert with other factors) by activating the genes for inducible nitric oxide synthase (iNOS), MHC class I molecules, and IFN-α/β (35, 42, 65). In turn, IFN-α/β (generated as a result of these events or more directly in response to replicating virus) may activate the IFN-α receptor complex and overlapping signaling components to activate additional antiviral genes, in-
including IRF-1 and Stat1 (24; M. Lo and M. J. Holtzman, unpublished observations). Thus a cascade of common signaling components enables IFN-γ to efficiently activate a subset of immune-response genes that are oriented toward several levels of antiviral defense. These include 1) antigen recognition and T cell costimulation as well as immune cell recruitment (ICAM-1); 2) antigen processing (TAP-1); and 3) amplification of the immune response (Stat1 and IRF-1) with additional capacity for antigen recognition (MHC class I) and antiviral toxicity (iNOS and IFN-α/β).

Based on the apparent efficiency of epithelial immune-response genes for antiviral defense, one might expect little problem in coping with respiratory viruses. However, this is clearly not the case, and these failures of the immune response provide another informative strategy for unraveling the genetic code of epithelial-dependent immunity. Thus viruses have established an array of strategies for immune subversion (108). In the case of riboviruses with relatively small genomes, molecular adaptation may take advantage of host cell machinery to subvert the immune response. In fact, ICAM-1 itself was discovered in part by its capacity to also serve as the receptor for the major group of human rhinoviruses (47, 123). Paramyxoviruses appear to block type I IFN signal-
ing (Lo and Holtzman, unpublished observations). Other viruses may encode proteins with the capacity for molecular mimicry that specially target IFN-γ-dependent immunity by modifying host gene expression. In particular, adenoviral E1A oncoprotein may interfere with p300/CBP action and may also directly target Stat1 (82). The molecular basis for this action and for how it can be used to develop anti-inflammatory strategies is discussed further in the section on strategies for correcting abnormalities in epithelial signaling and remodeling (sect. vi).

C. Virus-Dependent Gene Network: Posttranscriptional Regulation

Similar to the case for ICAM-1, only little was previously known for mechanisms that regulate RANTES gene expression (or β-chemokine gene expression in general) (6). On the basis of the standard approach noted above for defining cis- and trans-acting controls for gene transcription, initial studies by others suggested that RANTES gene expression depended on NF-κB sites in the RANTES promoter region (93, 100, 111). However, no attempt was made to determine the functional importance of these sites by direct determination of transcriptional initiation rate, and no information was available on any possible role of posttranscriptional regulation of the RANTES gene. This section summarizes our recent findings related to transcriptional and posttranscriptional regulation of the RANTES gene in airway epithelial cells using systems for IFN-γ- and virus-inducible expression in isolated cells. Later sections define how this system behaves in vivo.

In initial experiments (as noted above), we determined the capacity of primary culture airway epithelial cells to produce chemotaxins that could mediate T cell transmigration (68, 129). In this setting, we found that IFN-γ caused production of RANTES consistent with the IFN responsiveness of this cell type. However, we were interested in determining whether airway epithelial cells could respond directly to viral infection without a requirement for signals from immune cells. We were especially interested in whether the types of viruses associated with asthma might produce distinct effects on epithelial immune-response gene expression. The effects of paramyxoviruses appeared particularly relevant, based on the epidemiological evidence that members of the Paramyxoviridae family, especially respiratory syncytial virus (RSV) and parainfluenza viruses, were closely associated with recurrent wheezing and asthma in young children (19, 26, 107, 119, 122, 124). In that context, we were interested to find that RSV infection of human airway epithelial cells (using an ex vivo system lacking IFN-γ or other immune cell contribution) caused induction of RANTES gene expression in marked excess of other β-chemokine genes (69).

We next aimed to define the mechanism responsible for virus-inducible RANTES production. Previous analysis of the host response to viral infection has generally focused on the capacity of viruses to activate or repress transcription of cellular genes (52, 87), and this approach is also characteristic of work on riboviruses. In related examples from the Paramyxoviruses, the effect on host genes is mediated by DNA regulatory elements that bind NF-κB, IRF-1, ATF-2/c-Jun, and high mobility group protein HMG-I(Y) in the IFN-β gene or HMG-I(C) in the RANTES gene (78, 130, 132). Accordingly, we assumed that NF-κB sites in the RANTES gene promoter (93, 100, 111) might be responsible for virus induction of RANTES gene expression. Indeed, it initially appeared that RSV-driven expression of epithelial RANTES also depended on inducible gene transcription because expression was accompanied by coordinate increases in transcriptional initiation rate and gene promoter activity. However, RSV-driven increases in RANTES gene transcription and promoter activity were small and transient relative to RANTES expression, and they were no different in size and duration than for inactivated RSV that was incapable of inducing RANTES expression. These findings suggested that the increase in RANTES gene transcription was required but not sufficient for inducible expression and that critical regulatory effects occurred at a posttranscriptional level. This type of mechanism for virus-inducible expression of RANTES was established when we found that replicating RSV markedly increased RANTES mRNA half-life (69).

In contrast to what appear to be likely DNA-protein interactions for mediating transcriptional activation of host genes by viruses and other stimuli, little is known about viral mechanisms for controlling mRNA stability. In concomitant work on cytokine responsiveness of epithelial immune-response genes (including ICAM-1, IRF-1, and RANTES), we have noted that IFN (like RSV) stimulates RANTES production via mRNA stabilization (68). It is therefore possible that RSV-driven signals for altering stability of RANTES mRNA may overlap with those for IFN-γ signal transduction. However, the available examples for IFN-γ-dependent increases in mRNA stability appear distinct from the characteristics of RANTES expression. For example, IFN-γ stabilization of ICAM-1 mRNA is mediated by a region of the translated sequence encoding the ICAM-1 cytoplasmic domain (103), but the RANTES gene (encoding a secreted protein) does not contain this sequence. Furthermore, this instability mechanism (in contrast to the one for RANTES) is uninfluenced by actinomycin D treatment. Similarly, IFN-γ upregulates expression of the complement components C3 and C4 by stabilization of mRNA, but this system is also uninfluenced by transcriptional inhibition (91). In addition, the RANTES mRNA does not contain consensus sites for previously identified mRNA turnover elements, including AU-rich elements in other cytokine (as well as ICAM-1) mRNAs or UC-rich cleavage sites in gro α and 9E3 mRNAs.
(13, 103, 126, 127). Thus the mechanism underlying basal instability as well as RSV-dependent stabilization of RANTES mRNA may be biochemically distinct from other genes. Indeed, RNase protection assays of heterologous promoter/reporter plasmids indicate that basal instability of RANTES mRNA is mediated at least in part by nucleotides 11–389 of the RANTES gene, and this region is also the target for induction by virus (69). This region contains a distinct RNA turnover element in the 3′-untranslated region (UTR) that forms a complex with a putative RANTES-binding factor (RBF) under basal conditions but not during RSV infection (A. Antao, W. Roswit, M. Pelletier, and M. J. Holtzman, unpublished observations) (Fig. 4). The precise nature of this RNA-protein interaction and how it is regulated by viral replication still needs to be determined.

Even at this point, however, the findings provide the basis for an alternative model for virus-dependent induction of epithelial immune-response genes (depicted in Fig. 5). In this model, cytokine- (especially IFN-γ)-dependent induction of immune-response genes depends on transcriptional activation of gene expression, whereas direct viral induction of expression may be regulated by transcriptional or posttranscriptional events. Transcription may depend on viral interaction with Toll-like receptors (TLR) and activation of NF-κB dependent pathways (71, 132, 133). This type of regulation depends on viral surface proteins (e.g., RSV F protein) and may therefore be triggered by dead or live virus. In addition, viruses may also act downstream at the posttranscriptional level. This action requires viral replication and alters gene expression by stabilizing mRNA (which is the case for RANTES) or improving protein translation and processing (which appears to be the case for other epithelial immune-response genes). These two actions, activation of gene transcription and stabilization of mRNA, would be highly synergistic for gene expression. In fact, our experience has been that viral induction of epithelial gene expression is minimal in the absence of viral replication and consequent action at a posttranscriptional level (69; A. Antao, M. Pelletier, and M. J. Holtzman, unpublished observations).

D. The Final Common Pathway: Epithelial Cell Death

Each of the systems described above aims to explain how epithelial cells communicate with the immune sys-

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**FIG. 4.** Regulation of RANTES mRNA turnover under basal conditions and during viral infection. Under basal conditions, a trans-acting mRNA turnover factor (i.e., RBF) may be generated and activated and so be available to bind to a cis-acting mRNA turnover element in the 3′-untranslated region (UTR). RBF-mRNA interaction alters the conformation of the mRNA to render it susceptible to degradation by active endoribonuclease (RNase) at the degradation target site. During viral infection (e.g., by RSV), the RBF is prevented from binding (by downregulating the level or activation status) and from mediating RNase action. This mRNA stabilization may synergize with transcription to mediate marked increases in gene expression. [Modified from Holtzman et al. (53).]
tem. In the setting of viral infection, this communication is generally designed to recruit immune cells that destroy the infected host (epithelial) cells and then clear the cellular debris from the site. In this setting, cytotoxic T cells can mediate epithelial cell death via Fas-dependent and perforin/granzyme-dependent pathways (96), and macrophages may clear the debris from the site. We will return to the specifics of this process in section III, which defines the host response to viral infection in vivo, but at this point, we introduce the role of intrinsic pathways to regulate airway epithelial cell death and survival during the course of viral infection.

The approach to defining epithelial life and death pathways that are targeted during acute respiratory viral infection begins with the premise that the virus and the host have opposing motives. The virus aims to first maintain host viability (to allow for viral replication) and then trigger cell death (to allow for lysis and viral spread to adjacent cells). In contrast, the epithelial host aims to destroy itself as rapidly as possible, presumably via apoptosis, to protect its neighbors from infection and further inflammation. Thus self-programmed epithelial cell death is an effective innate host defense mechanism. For example, activation of caspase-dependent epithelial death pathways by the Fas death receptor may be critical for host defense against *Pseudomonas aeruginosa* infection in mice (45). However, we have found that primary culture human airway epithelial cells exhibit minimal Fas-dependent cell death unless the response is augmented by concomitant treatment with cycloheximide or actinomycin D, and paramyxovirus-inducible cell death is not influenced by Fas blockade (105). Thus the relevance of this system remains uncertain, especially in response to intracellular stimuli like respiratory viruses.

Recognizing that the pathways governing cell death versus survival are complex, we have analyzed the behavior of airway epithelial cells during viral infection using an oligonucleotide microarray in conjunction with assays of cell death parameters, e.g., caspase activation and mitochondrial dysfunction. Our preliminary results indicate that RSV infection causes mitochondrial dysfunction, but lethal effects appear to be delayed until viral replication is
well developed (M. O’Sullivan, K. Takami, and M. J. Holtzman, unpublished observations). The initial phase may rely on alterations in Bax-, TNF-, growth factor-, and cell cycle-dependent pathways that are each pushed toward gene expression that would favor cell survival during the acute phase of RSV infection (Fig. 6). Presumably, paramyxoviruses have developed a diverse program for preserving host cell viability during this early phase of infection, because their survival depends on it. Concomitant with these events, we also observe changes in Bcl-2-dependent pathways that would lead to mitochondrial dysfunction and cell death. Whether this change reflects host defense or the initiation of cell lysis by the virus will require additional work. In either case, an alteration in this step may provide a target for improving control of paramyxoviral infection, even before immune cells arrive at the scene. We still know little about how these life and death pathways may be altered in airway disease, but the indispensable role for them to govern cell growth and differentiation argues that they must be involved in pathogenesis. For example, preliminary evidence suggests that the Fas death pathway may be abnormally regulated in immune cells in asthma (61). In section V, we present experimental evidence for virus-inducible alterations in the epithelial repair process, and these alterations are also linked to the controls for epithelial cell death versus growth and differentiation.

III. AIRWAY IMMUNITY AND INFLAMMATION IN A MOUSE MODEL OF VIRAL INFECTION

Results from murine models of airway inflammation have been reported on numerous occasions (21, 22, 36, 43,
83, 97, 116, 121) and reviewed by us and others (28, 55–57). In the present context, we aimed to develop a mouse model to better determine how the airway epithelial system operates in vivo and to subject the system to genetic modification. Because the epithelial system is programmed for antiviral defense, we also aimed for a model with high fidelity to viral bronchiolitis in humans. Although RSV is often used for studies of human airway epithelial cells and human subjects, based on its link to childhood asthma, this virus does not cause a similar type of bronchiolitis in the mouse. For that reason, we chose another Paramyxoviridae family member, mouse parainfluenza type I or Sendai virus based on its capacity, as a natural pathogen in rodents, to cause top-down infection leading from the nose to the bronchi to the bronchioles to the alveoli. By limiting the inoculum, infection is limited to the airways and so resembles the pathology and pathophysiology of the human condition (136, 137, 139, 140).

This pathogenesis is the same as observed in human subjects with paramyxoviral infection and so offers an appropriate model for analysis. Sendai virus also infects isolated human airway epithelial cells, presumably because the allantoic fluid medium provides the Clara cell tryptase that is ordinarily provided by the rodent airway. Thus heterologous cell systems can be used to validate fidelity to RSV behavior when possible. This section summarizes our initial experience with this model for first identifying gene expression that is prominently inducible in airway epithelial cells during paramyxoviral infection and then determining the response in same-strain mice with targeted mutagenesis of these genes.

A. Epithelial Gene Expression: Interaction Between Virus and Host Cell

The initial step in defining epithelial cell-dependent immunity came with viral induction of epithelial immune-response genes in vitro. The second step came with induction of the same genes in vivo. In particular, we have found that paramyxoviral bronchiolitis causes induction of epithelial ICAM-1, Stat1, and RANTES gene expression in a pattern that is remarkably similar to the one observed in isolated airway epithelial cells. Accordingly, it appears that the mechanism for viral induction of epithelial immune-response genes in vivo may be similar to the post-transcriptional mechanism observed in vitro for RANTES. Thus, like RANTES, Stat1 gene expression is markedly upregulated in the epithelial host cell by Sendai viral infection in vivo (138), similar to the earlier experience with RSV infection of isolated human airway epithelial cells (T. Koga, D. Sampath, M. Lo, and M. J. Holtzman, unpublished observations). The sequence of the 5’-regulatory region of the mouse Stat1 gene shows a GAS consensus site for binding Stat1 homodimer in a location comparable to the human gene promoter region. However, this type of activation would require activation of Stat1 by IFN-γ, and we have found that IFN-γ-deficient mice exhibit the same virus-inducible expression of epithelial Stat1 in vivo. In contrast to human, there are no putative regulatory sites for binding ISGF3, NF-κB, or IRF-1 in the mouse Stat1 promoter. These findings suggest that paramyxovirus may directly alter Stat1 gene expression without a direct requirement for IFN- or NF-κB-dependent signaling. Consistent with these findings, experiments using isolated airway epithelial cells indicate that RSV causes little change in transcription rate of the Stat1 gene despite marked increases in gene expression (T. Koga, M. Pelletier, A. Antao, and M. J. Holtzman, unpublished observations). Additional definition of Stat1 (and other epithelial) gene expression is needed, but each of the available findings points to the possibility that viruses trigger the network of epithelial immune-response genes at a posttranscriptional level using a mechanism that is sensitive to viral replication.

Despite the uncertainty over the precise mechanism for viral regulation of epithelial gene expression, it is evident that the site and the timing of viral replication correlate closely with induction of expression. Thus both events are localized predominantly and coordinately to epithelial cells lining the airway. The entire profile of epithelial gene expression in this setting is still being defined by oligonucleotide microarray, but initial results continue to suggest close correlation between in vitro and in vivo changes in expression. As was the case in vitro, induction of epithelial gene expression in vivo also requires viral replication and is uninfluenced by preparations of inactivated virus. Furthermore, epithelial gene expression is followed closely by immune cell accumulation and activation at the site of viral infection. Each of these findings reinforces the proposal that the intrinsic response of the epithelial host cell to replicating virus may be critical for innate immunity, and so begs the question to more precisely define the role of the barrier epithelial cell in this setting. The next section describes our current understanding of epithelial function for host defense in the short term, and subsequent sections will define the implications of epithelial-viral interaction for a more chronic process.

B. Toward Epithelial Gene Knockouts: Defining an Active Role in Innate Immunity

This section summarizes the behavior of mice with targeted null mutations in selected immune-response genes in the setting of paramyxoviral infection. Because the tools are still being developed to achieve specific targeting, in airway epithelial cells and epithelial cell subsets (i.e., ciliated, Clara, goblet, or basal cells), the results
do not yet fully determine whether and which type of epithelial cell may be critical for innate immunity to respiratory viruses. Nonetheless, even the current evidence from the mouse model strongly favors the hypothesis that epithelial cells contain a specialized genetic program that is critical for antiviral host defense as catalogued below and summarized in Table 1.

1. IFN-γ and IL-12

The possibility that epithelial barrier cells might contribute to host defense first came from experiments that examined the role of immune cells. Thus the Th1-dependent response has been traditionally designated as critical for defense against respiratory viruses, and essential regulators of Th1 cell responses are IFN-γ and IL-12 (94). Accordingly, these cytokines might be expected to be derived from immune cells and be essential for host defense in the setting of viral infection. However, IFN-γ deficiency caused no significant change in the response to paramyxoviral infection (139). In particular, induction of epithelial immune-response genes proceeded at the usual level, indicating that viral effects on the epithelial cell did not depend on IFN-γ and instead might reflect direct actions of replicating virus within the host cell (as noted above for viral effects on epithelial cells in vitro). In addition, IFN-γ-deficient mice exhibited no defect in recovery from paramyxoviral infection, indicating that the epithelial program may be capable of directly helping with protection or for arming other aspects of the mucosal immune response.

The response of IL-12-null mice to paramyxoviral infection proceeded with a similar lack of immunocompromise but was even more informative for the role of the epithelium. Thus IL-12 is a heterodimeric protein consisting of p35 and p40 protein subunits. Initial results with null mutations targeted to the IL-12 p35, p40, or both IL-12 subunit genes produced mice that exhibited no defect in clearance of Sendai viral infection, indicating that IL-12 like IFN-γ production was also not essential for antiviral defense (137). Somewhat unexpectedly, however, mice with IL-12p35 deficiency exhibited increased airway inflammation (characterized by excessive macrophage accumulation) and increased mortality during infection. Because IL-12 is generally produced by antigen-presenting cells (i.e., macrophages, dendritic cells, B cells) (23, 84, 125, 135), we next defined the site of IL-12 induction and surprisingly found that expression was inducible by viral infection and was predominantly localized to airway epithelial cells. Initial IL-12 induction was followed by excessive expression of IL-12 p40 (often as homodimer IL-12p80) that could be further enhanced in IL-12 p35-deficient mice. Others have provided evidence that IL-12 p40 may function as an antagonist of IL-12 action (50, 67, 76, 152), but in the present case, its production was associated with increased mortality and epithelial macrophage accumulation. Although toxicity has been observed for overproduction of IL-12 (37, 112), inflammation due to IL-12 p40 had not been observed. Thus the results placed epithelial cell overgeneration of IL-12 p40 as a key intermediate for virus-inducible inflammation and as a candidate for epithelial immune-response genes that are abnormally programmed in inflammatory disease. As noted below, this possibility was further supported when we observed increased expression of IL-12 p40 selectively in airway epithelial cells in subjects with asthma and concomitant increases in airway levels of IL-12 p40 (as homodimer) and airway macrophages.

Taken together, these experiments with IL-12 suggest a novel role for epithelial-derived IL-12 p40 in modifying the level of airway inflammation during mucosal defense and disease. The results also serve to introduce a theme that will develop for each of the epithelial immune-response genes, i.e., the usual level of epithelial gene expression may aid in host defense, whereas excessive expression may lead to inflammatory disease. In this case, epithelial IL-12 p80 production may help to mediate macrophage recruitment and/or activation, whereas higher or inappropriate levels of production may lead to macrophage-dependent inflammation. This view favors cellular recruitment as a feature of host defense, but from the viral perspective, recruitment may facilitate viral spread
to additional cell populations, including neighboring epithelial cells that may also respond to chemokatins.

2. **ICAM-1**

ICAM-1 is a predominant determinant of epithelial-immune cell adhesion, and transmigration in vitro and epithelial expression is prominently inducible by paramyxoviral infection in vitro and in vivo. Thus mutagenesis of the ICAM-1 gene was a natural target to influence airway inflammation in paramyxoviral bronchiolitis. As might be predicted, ICAM-1-null mice are indeed protected from airway inflammation (as assessed by accumulation of immune cells in airway tissue) induced by paramyxoviral infection compared with same-strain controls (136). The blunted inflammatory response in ICAM-1-null mice was accompanied by less efficient viral clearance but was still beneficial to the host, since this cohort experienced less weight loss after bronchiolitis and lower mortality rates after a larger inoculum that causes bronchopneumonia.

We have long proposed that airway inflammation may lead to airway hyperreactivity (53, 54), but definitive proof has been difficult to obtain through pharmacological and physiological approaches. Accordingly, we next determined if ICAM-1-null mice that are relatively protected from virus-induced airway inflammation are also protected from postviral hyperreactivity. Indeed, using whole body barometric plethysmography to measure enhanced pause (Penh) as an index of airway obstruction at baseline and after methacholine challenge in wild-type and ICAM-1-null mice, we found 1) slightly increased baseline and reactivity by 1 wk postinoculation that was partially blocked in ICAM-1-null mice, and 2) normal baseline but markedly increased reactivity by 3 wk after inoculation that was completely blocked in ICAM-1-null mice. As noted above, the predominant site of ICAM-1 expression during paramyxoviral bronchiolitis is the airway epithelial cells that are home to the virus, but whether this cellular site is the one responsible for downregulating inflammation and hyperreactivity will require more cell-specific targeting. As discussed further in section v, genetic susceptibility to virus-inducible hyperreactivity can also be defined in this system, since only certain strains of inbred mice develop the acute inflammation/hyperreactivity phenotype as well as the subsequent and persistent remodeling response.

3. **RANTES**

Experimental paramyxoviral bronchiolitis in mice causes marked induction of RANTES gene expression in the lung, and expression is localized predominantly to airway epithelial cells and to adjacent tissue macrophages (N. Kajiwara, M. J. Walter, M. O’Sullivan, J. Tyner, O. Uchida, D. N. Cook, Y. Makino, T. M. Danoff, and M. J. Holtzman, unpublished observations). This pattern correlates precisely with the location of viral replication and so fits closely with findings in humans with paramyxoviral infection and in isolated cells, indicating that viral replication is a potent and direct inducer of RANTES gene expression (69). Additional experiments indicate that mice with targeted disruption of the SCYα5/RANTES gene are immunocompromised to the point of overwhelming viral infection and death (Kajiwara et al., unpublished observations). This defect appears to be manifest because RANTES is required to block apoptosis of virus-infected macrophages. The physiological source of RANTES in vivo could be either the epithelial cell or the macrophage, so these experiments did not yet add to the primary role of the airway epithelial cell in this setting. Nonetheless, this chemokine function is distinct from ones that have been previously identified in the setting of infection, such as recruiting and activating immune cells, interfering with viral entry receptors, or triggering cell death receptors, and so establish a novel mechanism for host defense based on preserving viability of the infected macrophage via a distinct combination of antiapoptotic and antiviral actions of a chemokine. Thus defense against intracellular pathogens, particularly viruses, depends on programmed death of infected host cells and then clearance of these cells by phagocytic macrophages. For effective clearance to take place, the viability of macrophages must be maintained in the face of infection, and these results suggest a molecular basis for preservation of virus-infected macrophages. The precise molecular mechanism for RANTES to influence cell death pathways still needs to be determined, but initial observations indicate that signaling to the death pathway proceeds via specific chemokine receptors that are also susceptible to regulation by viral replication (J. Tyner, O. Uchida, and M. J. Holtzman, unpublished observations). However, the results with RANTES- and IL-12-deficient mice reinforce another theme of this system, i.e., both epithelial and macrophage components of the innate immune response are critical targets for the virus and for effective antiviral defense.

4. **Stat1**

Stat1 mediates the expression of a subset of interferon-inducible genes (typified by ICAM-1) in airway epithelial cells, so Stat1 deficiency should have a major impact on epithelial function during viral infection. Indeed, Stat1-null mice exhibit markedly increased weight loss and decreased survival after Sendai viral infection (118). Thus intranasal inoculation with low levels of Sendai virus causes little effect in same-strain control mice but 100% mortality in Stat1-null mice. These results are similar to reports of Stat1-null mice in other viral models, but the basis for the defect in host defense has not been determined (29). In the present setting, Stat1 deficiency is associated with marked increases in viral replication rates and severe airway inflammation.
with cellular infiltrate and debris in the lumen. This host response may not simply reflect increased viral tissue load, since the response is not observed in wild-type mice even at a high inoculum that results in comparable viral load and levels of mortality. The luminal infiltrate is comprised mainly of neutrophils that contain virus and exhibit apoptosis and is fully manifest even at 8 days after infection. Because neutrophils are ordinarily cleared by this time in wild-type mice (ordinarily the responsibility of activated macrophages), the results suggest a possible delay in neutrophil clearance in Stat1-null compared with wild-type control mice.

These findings suggested that Stat1 in epithelial cells (leading to enhanced viral replication) or macrophages (leading to decreased activation) might underlie the defect in antiviral defense. Additional experiments with bone marrow radiation chimeras were used to dissect the role of Stat1 in the radiation-resistant compartment (especially the airway epithelium) versus radiation-sensitive hematopoietic cells (including macrophages). In this case, we found that lethally irradiated Stat1-deficient mice reconstituted with wild-type bone marrow were still susceptible to infection with Sendai virus, whereas wild-type mice that received Stat1-deficient bone marrow retained resistance to virus (L. Shornick, D. Briner, M. Lo, and M. J. Holtzman, unpublished observations). The viral susceptibility of chimeras with Stat1-deficient epithelium exhibited the same pattern of persistent luminal inflammation. Taken together, the results suggest that epithelial Stat1 may be critical for antiviral defense, perhaps by limiting viral replication and so aiding the removal of virus-infected, apoptotic neutrophils.

Because Stat1-deficient mice may have a selective and profound defect in interferon signaling (30, 88) and IFN-γ-deficient mice exhibit no significant immunocompromise with Sendai viral infection, the findings implicate Stat1-dependent effects of IFN-α/β signaling as critical for antiviral defense against respiratory paramyxoviral infection. However, related experiments in isolated airway epithelial cells indicate that paramyxoviruses downregulate IFN-α/β signaling as part of a strategy to establish infection (M. Lo and M. J. Holtzman, unpublished observations). Whether further decreases in this pathway or in other pathways lead to Stat1-deficient immunocompromise in vivo still needs to be determined. Defining the mechanism for Stat1-dependent protection in paramyxoviral bronchiolitis and the particular role of epithelial (versus immune cell) function of Stat1 may finally define a primary and distinct role of the epithelial barrier cell in innate immunity.

IV. AIRWAY IMMUNITY AND INFLAMMATION IN HUMAN SUBJECTS WITH ASTHMA

On the basis of the rationale that an abnormal immune response is part of inflammatory disease, it appeared reasonable to next determine the behavior of epithelial immune-response genes in asthma. Initial studies indicate that airway epithelial cell expression of Stat1 and its target genes as well as IL-12 p40 and RANTES are all altered in asthma, and this section summarizes these findings. Because each of these epithelial immune-response genes are highly responsive to paramyxoviral infection (with or without concomitant production of IFN-γ), their activation in asthma brings into question the hypothesis that asthma develops due solely to decreased Th1- and increased Th2-type T cell responses. This issue is also discussed in this section.

A. Constitutive Abnormalities in Epithelial Gene Expression (Stat1 And IL-12)

Cytokine effects on immunity and inflammation often depend on STAT signaling pathways, so these are ideal candidates for influencing inflammatory disease. We reasoned that selective interferon responsiveness of the first STAT family member (Stat1) and Stat1-dependent immune-response genes such as ICAM-1, IRF-1, and Stat1 itself in airway epithelial cells provided a basis for detecting cytokine signaling abnormalities in inflammatory airway disease. Based on nuclear localization and phosphorylation, epithelial Stat1 (but not other control transcription factors such as Stat3, AP-1, and NF-κB) was invariably activated in asthmatic compared with normal control or chronic bronchitis subjects (114). Stat1 activation was relatively selective for epithelial cells, since activation was not detectable in neighboring macrophages. Furthermore, epithelial levels of activated Stat1 correlated with levels of expression for epithelial ICAM-1, IRF-1, and Stat1, and in turn, ICAM-1 level correlated with T cell accumulation in tissue. However, only low levels of IFN-γ or IFN-γ-producing cells were detected in airway tissue in all subjects. Since tissue levels of IFN-γ are not increased, asthma appears to be characterized by constitutive activity of Th1-like cytokine signaling even without evidence of stimuli (such as IFN-γ or viral infection) (69, 138) that normally drive this type of immune reaction. The results therefore provide initial evidence linking abnormal behavior of STAT pathways for cytokine signaling (as opposed to cytokine production) to the development of an inflammatory disease. In that context, the results also change the current scheme for asthma pathogenesis to one that must include a localized gain in transcriptional signal ordinarily used for a Th1 cytokine (IFN-γ) and so is distinct from mechanisms that depend on allergy-driven overproduction of Th2 cytokines.

Localizing the abnormality in Stat1 activity to airway epithelial cells is critical to the impact of these findings. Constitutive STAT activities in lymphocytes are associated with malignant transformation, whereas excessive
Stat1 activity in chondrocytes driven by a mutant fibroblast growth factor receptor may be associated with growth arrest (128). Thus depending on the programmed cytokine response of specific cell types in specific tissues, abnormal Jak-STAT activity may lead to diseases as diverse as lymphoma or dwarfism. Consequently, analysis of cytokine-dependent responses in asthma using sites distant from the airways may not reflect behavior in airway cells. For example, others have indicated that decreased Th1 responses may predispose to asthma based on an association with diminished delayed-type hypersensitivity reactions to tuberculin (117). However, this association was based on immune responses in the skin and so does not reflect the specific activity of Th1-driven signaling pathways in airway epithelial cells.

To next determine whether the pattern of IL-12 upregulation in murine viral bronchitis also develops in asthma, we measured levels of IL-12 expression in airway tissue and bronchoalveolar lavage fluid obtained from asthmatic subjects. In fact, subjects with asthma exhibited higher levels of IL-12 as well, with expression predominantly in airway epithelial cells and in the form of IL-12 p40 (and p80 homodimer). Upregulation of IL-12 p40 expression also correlated with increased number of macrophages in the airway, so each of the features of IL-12 behavior was similar to ones found in the mouse during paramyxoviral infection. This finding offers two new possibilities for a role of IL-12 p40 in asthma: 1) antagonism of endogenous IL-12, and so skewing the local cytokine environment toward a Th2 immune response, and/or 2) function as an agonist, e.g., as a macrophage chemotactic and activating factor, and so causing airway inflammation. In fact, some (but not all) previous studies find significant macrophage accumulation in the submucosal and intraepithelial airway tissue of asthmatic compared with normal subjects (104, 109), whereas others provide evidence of macrophage activation in asthma (18) as well as an increase in number and activation state of airway macrophages during allergen challenge in asthma (89). In either case, these results provide initial evidence that asthma, often characterized as a condition that depends on overexpression of Th2 and underexpression of Th1 cytokines by immune cells, does in fact also exhibit overexpression of IL-12 p40 that appears to be chiefly derived from airway epithelial cells. In conjunction with previous observations of constitutive activation of Stat1 and Stat1-dependent genes, the findings further support the possibility that pathways normally responsive to Th1 cytokines are also dysregulated in airway inflammatory disease. As discussed further below, upregulation of epithelial Stat1 and IL-12 pathways in asthma is found in both allergic and nonallergic subjects and with or without treatment with glucocorticoids.

B. Glucocorticoid-Sensitive Abnormalities in Epithelial Gene Expression (RANTES)

Recognizing that RANTES and Stat1-dependent genes exhibit distinct but complementary function (chemotaxis versus cell adhesion) and regulation (posttranscriptional versus transcriptional), we also determined the level of epithelial RANTES expression in asthma. Most previous reports indicated that RANTES is expressed at similar basal levels in the airway epithelium of normal versus asthmatic subjects with mild disease (8, 33, 60, 144). Similarly, we did not expect much change in epithelial RANTES levels in response to allergen, based on our experience with segmental allergen challenge (M. Castro and M. J. Holtzman, unpublished observations). Accordingly, we sought an experimental protocol for endogenous exacerbation of asthma that might better reflect natural flares of the disease and might better define the mechanism of action of anti-inflammatory treatment.

In that context, we developed a protocol for controlled glucocorticoid withdrawal in stable asthmatic subjects that includes measurements of airway function and endobronchial biopsy and lavage during treatment and then after withdrawal of inhaled glucocorticoids (15). In some subjects, glucocorticoid withdrawal results in asthma exacerbation characterized by increases in airway hyperreactivity and immune cell (predominantly T cell) infiltration. Interestingly, these disease flares are consistently associated with increased expression of RANTES (by in situ hybridization for RANTES mRNA) that appears localized predominantly to airway epithelial cells (129; M. Castro, S. Block, D. L. Hamilos, M. V. Jenkersen, Q. Li, T. Horiuchi, K. Schechtman, and M. J. Holtzman, unpublished observations). Concomitant work has indicated that there is no increase in IFN-γ production or NF-κB activation in this setting, similar to the findings in stable asthma. Thus epithelial RANTES production is inducible during asthma exacerbation even in the apparent absence of viral infection or IFN-γ production. Furthermore, induction is sensitive to glucocorticoid treatment but appears to proceed via an NF-κB-independent pathway. These findings are analogous to those in paramyxoviral infection, so it is possible that posttranscriptional events underlying RANTES expression during paramyxoviral infection may also be relevant to expression in asthma. As discussed in section IV, we have proposed that the increased expression of each of these pathways, i.e., Stat1, IL-12 p40, and RANTES, may reflect a response to virus that no longer causes signs of infection, but in the case of RANTES, expression is unmasked by glucocorticoid withdrawal. Defining the basis for induction of RANTES gene expression and its functional implications will require additional work on the molecular basis for RANTES expression in isolated cell and animal model systems as well as correlative protocols in human subjects. For example,
glucocorticoid effects on posttranscriptional regulation of the RANTES genes still need to be determined. Nonetheless, the findings for Stat1, IL-12, and RANTES already suggest that a propensity for epithelial gene expression may be conditioned by viral exposure and so underlie the relationship of childhood viral infection to ongoing asthma even later in life.

C. A Revised Model for Th1/Th2 Contributions to Asthma

Asthmatic inflammation has been attributed to an abnormal sensitivity to inhaled allergens, and by extension, to a skewed T helper cell response with increased Th2 and decreased Th1 components compared with normal. In some settings, Th1 cells (perhaps activated by viral infection) may assist Th2 cells in initiating an allergic response, but this scheme also relies on Th2-dependent production of cytokines, notably IL-4, IL-5, and IL-13 to drive asthmatic inflammation and consequent pathophysiology (as summarized in Fig. 1). However, this pathogenesis does not account for the discrepancy between allergic and asthmatic phenotypes and, as indicated in the previous section, does not provide for the heightened antiviral state of the airway epithelium in asthma. In particular, increased activity of innate antiviral programming in the epithelium appears to be a fundamental feature of asthma. This alternative pathway is normally useful for airway barrier cells to defend against respiratory viruses, but if overactive, could lead to inflammation, as appears to be the case in asthma. Some epithelial programs appear insensitive to glucocorticoids and so are present constitutively even in stable disease (i.e., Stat1, IL-12 p40 networks), whereas others are sensitive to glucocorticoids and so inducible by glucocorticoid withdrawal and flares of the disease (i.e., RANTES). As perhaps expected for an antiviral system, this epithelial network is generally oriented toward a Th1 response.

To better integrate these concepts and so better explain the pathogenesis of airway disease, we have revised the model for the role of the airway immune response in asthma. The revised model accounts for how an alternative Th1-oriented epithelial network may act in combination with an enhanced Th2 response, and the combination of epithelial, viral, and allergic components led to its designation as an Epi-Vir-All paradigm (Fig. 7). In this scheme, increases in epithelial antiviral signals (e.g., Stat1 activation and IL-12 p40 expression) and allergen-driven

![Figure 7](http://physrev.physiology.org/)

**Fig. 7.** Revised model for the role of the airway immune response in the development of airway inflammation and remodeling. A: illustration of how increases in epithelial antiviral signals (e.g., Stat1 activation and IL-12 p40 expression) and allergen-driven production of Th2 cytokines (e.g., IL-4, IL-5, and IL-13) are characteristic of subjects with asthma (designated as an “A”) studied under stable conditions during treatment with inhaled glucocorticoids. B: illustration of how further increases in epithelial signaling (driven by viral infection) or Th2 cytokine production (driven by further allergen exposure) may develop in subjects with asthma studied during a flare without glucocorticoid treatment. The abnormality in epithelial cell behavior may depend on persistence of virus at low copy number and proper genetic background. The combination of epithelial, viral, and allergic components led to designation of this pathogenesis model as an Epi-Vir-All paradigm.
production of Th2 cytokines (e.g., IL-4, IL-5, and IL-13) are characteristic of subjects with asthma under stable conditions during treatment with inhaled glucocorticoids. Further increases in epithelial signaling (driven by viral infection) or Th2 cytokine production (driven by further allergen exposure) would develop in subjects with asthma during a flare of the disease. In addition, increased levels of Stat1 may mediate a hypersensitive Th1-type response in the airway. At least in vitro, high levels of Stat1 are capable of priming the Stat1-dependent pathway and so lead to exaggerated gene expression in response to normal levels of stimulation (D. Sampath, Y. Zhang, and M. J. Holtzman, unpublished observations). In addition, flares of the disease are closely associated with induction of RANTES gene expression. This system, which appears to be regulated at the posttranscriptional level during viral infection, may rely on a similar regulatory mechanism in asthmatic flares. Each of these possibilities begs the question as to how the abnormality in epithelial cell behavior originally develops. In section V, we summarize initial evidence that viral infection and the genetic background of the host may interact to permanently reprogram the behavior of airway epithelial cells.

V. LONG-TERM AIRWAY HYPERREACTIVITY AND REMODELING IN MICE AND HUMANS

A relationship between viral infection and the development of chronic inflammatory disease has been proposed for diverse clinical syndromes, but the basis for this relationship is still uncertain. In the particular context of asthma, paramyxoviral infections are the leading cause of lower respiratory tract illness in infants and young children (19, 26), and children with clinically significant viral bronchiolitis appear to be marked for the subsequent development of a chronic wheezing illness that is independent of allergy (107, 119, 124). Presumably paramyxoviral infection triggers a switch to an abnormal host response, since paramyxoviruses (or other respiratory RNA viruses) are not thought to persist in airway tissue as a cause of chronic respiratory disease (1). With or without viral persistence, however, the role of specific host factors in the development of chronic wheezing or life-long asthma has not been determined. This section describes our initial results from studies of mice and human subjects in defining the linkage between respiratory viral infection and persistent asthma and in so doing extends the concepts of the previous sections that were focused predominantly on short-term immunity and inflammation.

A. Segregating Acute From Chronic Phenotypes

To better define viral and host factors in the development of the asthma phenotype, we took further advantage of the mouse model of paramyxoviral bronchiolitis. As noted above, inhibition of the acute inflammatory response could be achieved by disruption of epithelial immune-response genes. On the basis of the work in vitro, this epithelial gene network is directly inducible by viral replication and is dominated by an array of interferon-responsive genes, but among candidates that might mediate immune cell traffic, ICAM-1 appears to be a predominant determinant for immune cell transmigration (69, 82, 129). In fact, as noted above, we found that ICAM-1 is inducible primarily on host airway epithelial cells by viral infection and is necessary for full development of acute inflammation and concomitant postviral airway hyperreactivity that peaks at ~3 wk after infection in the mouse model. Unexpectedly, however, as we followed wild-type and ICAM-1-null mice in a homogeneous genetic background, we also found that primary viral infection caused essentially permanent airway hyperreactivity and concomitant epithelial remodeling that were manifest despite ICAM-1 deficiency and were not accompanied by low-level persistence of viral transcripts (136, 140; M. J. Walter, J. D. Morton, N. Kajiwara, E. Agapov, T. Horiiuchi, M. Castro, and M. J. Holtzman, unpublished observations). The predominant features of this remodeling phenotype, i.e., goblet cell hyperplasia and mucin production, are also inducible in human subjects with asthma. Taken together, the results indicate that paramyxoviral infection may cause both acute airway inflammation/hyperreactivity and chronic airway remodeling/ hyperreactivity phenotypes that can be segregated by their dependence on ICAM-1 and time and so may depend on distinct genetic controls (as modeled in Fig. 8). In a more general context, the findings establish the capacity of a single paramyxoviral infection to cause both acute and chronic manifestations of the phenotype for hypersecretory disease and the relevance of specific host defense genes in moderating the acute but not necessarily the chronic phenotype.

B. Genetic and Viral Determinants for Persistence

The striking possibility that viral infection at an early age may lead to permanent alterations in airway epithelial behavior and airway function requires significant further investigation. Initial results indicate that both viral and host factors may determine the outcome of infection. Thus only specific strains of mice are susceptible to developing the chronic phenotype, analogous but distinct from the diversity in resistance to acute infection (10; J. D. Morton and M. J. Holtzman, unpublished observations). Mouse age also affects the severity of the phenotype, indicating that developmental factors also influence the outcome. Improvements in the technology for genetic mapping (using single-nucleotide polymorphisms as informative markers) and gene expression (using oligonucle-
otide microarray) will allow more precise definition of epithelial programming that is responsible for asthma and bronchitis phenotypes in the mouse model (39). Extension of these results to analysis of candidate genes in human subjects using precise case-control studies may then provide further insight into the genetic susceptibility for virus-inducible disease in humans.

Although genetic susceptibility for the host response has been the target of studies by our lab and others, it appears likely that viral genetics are also critical to the development of the chronic remodeling/hyperreactivity phenotype. Thus previous studies of Sendai virus and related paramyxoviruses indicated that viral particles are normally eliminated from tissue after 10–12 days (25). However, these studies relied on relatively insensitive methods that are responsive to replicating (nonmutant) forms of virus, and recent studies of other riboviruses indicate that they may persist in the tissue as mutant quasi-species. In these cases, viral persistence may be facilitated by high mutation rates at critical epitopes and consequent escape from immune surveillance (27), but whether persistence is necessary or sufficient for chronic alterations in host cell behavior still needs to be determined. Initial analysis of Sendai viral titers using kinetic PCR suggests that viral persistence in host tissues at low copy number is not a common feature of paramyxoviral infection (J. D. Morton, E. Agapov, D. Palamand, and M. J. Holtzman, unpublished observations). Moreover, initial results with the Sendai viral mouse model indicate that viral persistence is not necessary or sufficient for the chronic phenotype, since sensitive and resistant strains of mice exhibit similar effectiveness in viral clearance. The results suggest a hit-and-run hypothesis for the viral effect, i.e., transient infection causes permanent alteration in host cell behavior. Even so, however, the viral gene products responsible for triggering the change in host phenotype still need to be defined. New systems for recovery of negative-strand RNA viruses entirely from cDNA may allow better definition of these issues as well as general understanding of replication and pathogenesis for this order of viruses (20, 95).

VI. SMART STRATEGIES FOR CORRECTING EPITHELIAL INFLAMMATION AND REMODELING

The airway epithelium (by virtue of location) is directly accessible to inhaled agents, so modifying epithelial function is a natural target for therapy of asthma and for combating respiratory viral infection. In the case of asthma, even current agents (such as glucocorticoids and sodium cromoglycate) may owe part of their efficacy to the attenuation of cell adhesion molecule and chemoat-
tractant function (as noted above for RANTES) and so could target the epithelium to achieve therapeutic effects. Similarly, delivery of blocking antibodies, soluble ligands, or synthetic peptide analogs via the airway may indicate the importance of local epithelial events if the reagents cannot effectively reach subepithelial sites (34, 49, 75, 86, 101). Using the epithelial antiviral network as a model, we have begun to develop more specific and perhaps smarter strategies for modifying epithelial gene expression and so influencing epithelial function. The approach derives directly from the molecular pathways defined in this review and is summarized in Figure 9. The overall aim is to improve airway disease, with special reference to asthma and related phenotypes in other forms of bronchitis/bronchiolitis. In view of the proposed nature of epithelial programming against respiratory viruses, another natural therapeutic target is to improve antiviral host defense.

A. Reversing Viral Mimicry Using Mutant E1A Oncoprotein

Immunity to viral pathogens depends on coordinated control of host cell genes, so viruses (including adenoviruses) have developed strategies to redirect the normal genetic program (108). As one of the most informative examples, the adenoviral early region 1A (E1A) gene encodes for an oncoprotein that activates host cell cycle and so enables DNA synthesis needed for viral replication. To accomplish this, E1A oncoprotein presents two distinct binding sites for competing with cell cycle regulators: one site uses conserved regions 1 and 2 (CR1 and CR2) to bind a family of antioncoproteins typified by retinoblastoma (Rb) protein; the other site uses NH2-terminal residues and CR1 to bind a family of transcriptional coactivators typified by p300 (92, 148) (Fig. 10). The Rb protein and Rb-related p107 and p130 proteins normally bind to the E2F transcription factor and silence genes needed for progression through the cell cycle (147). The p300 protein (17) and the related cAMP response element binding protein (CREB)-binding protein (CBP) (31) serve as adaptor proteins that link transcription factors such as CREB (3, 72) as well as p53 antioncoprotein (48, 74) to the basal transcription complex. In addition, p300/CBP proteins influence gene transcription by intrinsic histone acetyltransferase activity and by linking additional coactivators (such as p300/CBP/cointegrator-associated protein or p/CIP) to core histones (134). Thus, by sequestering (and perhaps linking) Rb- and p300/CBP-type proteins in competition with endogenous transcription factors and coactivators, three E1A domains (NH2 terminus, CR1, and CR2) are sufficient for mediating cellular proliferation (143).
In addition to altering control of the cell cycle, adenoviruses also aim to subvert host defense by evading immune detection. Early studies in cell lines suggested that E1A might inhibit activation of immune-response genes by disrupting events (such as protein phosphorylation) that lead to protein-DNA interactions at interferon-responsive enhancers (64). However, subsequent studies demonstrated that E1A capacity for immune suppression might also depend on targeting p300/CBP. Thus p300/CBP coactivator function was extended to c-fos and c-Jun components of activator protein-1 (3, 5), the p55 component of NF-κB (40), steroid and nuclear-hormone receptors (70, 150), and to the first two members of the STAT family (9, 58, 153) that mediate interferon-driven gene activation. In all cases, including Stat1 and Stat2, E1A influenced host gene expression by competing with these endogenous factors for binding to p300/CBP. Because Stat1 is critical for IFN-γ-driven gene transcription, competition for p300/CBP by E1A may underlie its capacity to subvert IFN-γ-stimulated immunity.

In reviewing this previous work, we questioned whether E1A might also act directly on a specific DNA-binding transcription factor and thereby provide determinants for more precisely influencing gene expression. Precedent for this possibility may be found in interactions of the transactivating domain of E1A (conserved region 3) with transcription factors needed for viral gene expression (77), but at least to date, E1A NH2-terminal interactions appear more highly restricted (92). Nonetheless, additional interactions might not be detected if E1A determinants for binding p300/CBP overlapped with those for binding more specific cellular activators. In addition, previous studies of adenoviral infection and host gene behavior often used transformed cell lines rather than the natural adenoviral host cell and so could not exclude confounding effects of other oncogenes (such as SV40 large T antigen) on E1A action (32).

In that context, we have used primary culture hTBE cells (along with endobronchial tissue and mouse models of viral bronchitis) to define epithelial cell-dependent im-
munity to respiratory pathogens (129, 138). Our work on isolated cells indicates that a subset of epithelial immune-
response genes typified by ICAM-1 are controlled by an IFN-γ-responsive Jak-STAT signaling pathway that relies
on Stat1 to first be phosphorylated at the IFN-γ receptor
complex and then to bind to the ICAM-1 promoter region
(80, 81, 141). At the promoter, Stat1 interacts with adja-
cent transcription factors such as Sp1 and with p300/CBP
(58, 81, 153) to facilitate enhanceosome formation and
induce gene transcription. As noted above, E1A-p300/CBP
interaction may therefore underlie its capacity to subvert
IFN-γ-driven gene activation, or alternatively, may ex-
plain the antiviral effect of IFN-γ (153).

Recently, however, we demonstrated that E1A can
also modify Stat1-dependent gene activation by direct
action on Stat1 itself (82). Using mutant forms of E1A, we
defined an NH₂-terminal determinant (Arg⁵) for binding
p300/CBP that is not needed for binding Stat1. Furthermore,
we showed that mutant E1A with no capacity for
binding p300/CBP or inhibiting p300/CBP-dependent
events (73, 143, 148) still binds Stat1 and inhibits IFN-γ-
induced, Stat1-dependent transactivation of exogenous
and endogenous target genes early in the course of ad-
enviral infection. This effect is distinct from downregu-
lation of Stat1 phosphorylation that occurs later in the
course of infection. Taken together, these results provide
for an overall scheme for adenviral disruption of host
gene expression during early and late-phase infection, a
novel action of the E1A NH₂-terminus in mediating im-
mune suppression, and a revised model of Stat1-depend-
ent gene expression. The results also provide an appro-
priate E1A-mediated strategy for more selectively
downregulating Stat1-dependent events in the airway.

B. Modifying Epithelial Signaling
With Mutant Stat1

Transcription factors generally contain at least two
independent domains for DNA binding versus activation
of transcription (44). Removal of the transactivation do-
main has been shown in many cases to result in an
inactive factor that can bind to the DNA element and
displace wild-type protein, thereby creating a dominan-
tive negative action (51). Targeting such a dominant-negative
construct so that it is expressed in a specific tissue has
been useful in understanding the function of specific tran-
scription factors in different tissues. Accordingly, this
strategy offers an advantage over complete deletion of the
factor by homologous recombination with the endoge-
nous gene if there is a goal of defining function in a
specific tissue or cell type. The creation of a dominan-
tive-negative mutation for Stat1 is more challenging than for
transcription factors with less complex function, but also
offers an opportunity for dissecting the relative impor-
tance of Stat1 modular function.

Accordingly, we aimed to selectively downregulate
the pathway using a dominant-negative strategy for inhi-
bition of epithelial Stat1 in the primary culture airway
epithelial cell model (141). In initial experiments using a
Stat1-deficient cell line, we demonstrated that transfection
of wild-type Stat1 expression plasmid restored ap-
propriate Stat1 expression and IFN-γ-dependent phos-
phorylation as well as consequent IFN-γ activation of
cotransfected ICAM-1 promoter constructs and endoge-
nous ICAM-1 gene expression. However, mutations of
Stat1 at Tyr-701 (the Jak kinase phosphorylation site),
Glu-428/429 (the putative DNA-binding site), His-713 (the
splice-site resulting in Stat1β formation), or Ser-727 (the
mitogen-activated protein kinase phosphorylation site) all
decreased Stat1 capacity to activate the ICAM-1 pro-
moter. The Tyr-701 mutant (followed by the His-713 mu-
tant) was most effective in disabling Stat1 function and in
overcoming the activating effect of cotransfected wild-
type Stat1 in this cell system, thereby highlighting the
effectiveness of blocking Stat1 homo- and heterodimer-
ization. In experiments using primary culture airway epi-
thelial cells and each of the four Stat1-mutant plasmids,
cotransfection with the Tyr-701 and His-713 mutants again
most effectively inhibited IFN-γ activation of an ICAM-1
gene promoter construct. By then transfecting airway
epithelial cells with wild-type or mutant Stat1 tagged with
a Flag-reporter sequence, we used dual immunofluores-
cence to show that cells expressing the Tyr-701 or His-713
mutants but not the two other Stat1 mutants or wild-type
Stat1 were prevented from expressing endogenous
ICAM-1 in response to IFN-γ treatment. The results pro-
vide the initial indication that loss of function may corre-
late with dominant-negative activity for Stat1 in a biolog-
ically relevant human cell model. The capacity of specific
Stat1 mutations to exert a potent dominant-negative ef-
fect on IFN-γ signal transduction provides for further
definition of Stat1 structure/function. The strategy also
provides a means for natural or engineered expression of
mutant or truncated Stat1 to selectively downregulate
dependent activity of this pathway in a cell type- or tissue-specific
manner during immune and/or inflammatory responses in vivo.

C. Future Considerations

The studies summarized above indicate that molecular
strategies can be devised that selectively target epite-
phelial signaling pathways. Thus downregulation of epithelial
Stat1 signaling (to correct the abnormality in asthma) and
possibly upregulation of RANTES signaling (to aid in
antiviral defense) may be devised from the regulatory
features of these pathways, and these strategies appear
effective in vitro. Studies in animal models are poised to
determine whether modifying epithelial immune function
may be effective as a sole treatment for airway inflammation or may render other anti-inflammatory drugs more effective in vivo. However, the efficacy of this approach will depend on developing methods to achieve selective and efficient expression in the airway epithelium. Thus proof of concept may be feasible using transgenic animals (limited only by specificity of available gene promoter systems for epithelial expression), but vectors with reproducible, sustainable, and high-level expression in the airway epithelium still need to be developed.

VII. SUMMARY

This review summarizes increasing evidence that airway epithelial barrier cells actively mediate airway immunity and inflammation. In fact, the role of the epithelium has evolved from a relatively simple scheme for mediating leukocyte recruitment to one that depends on the coordinated expression of a network of epithelial immune-response genes coordinated for host defense under distinct transcriptional and posttranscriptional controls. The transcriptional program is typified by an interferon-driven Jak/Stat signaling pathway, while posttranscriptional regulation uses RNA-protein interactions that are responsive directly to viral replication. Respiratory viruses ordinarily interact with this Th1-style gene network in a battle of host defense versus immune subversion, but the same network is also activated in asthma even in the absence of overt viral infection. Thus the barrier epithelial cell population appears specially programmed for normal host defense but abnormally programmed in inflammatory airway disease. Recent results indicate that airway epithelial cells may be reprogrammed for permanent proliferation and skewed mucus cell differentiation by asthmagenic RNA viruses that persist at low copy number. This alteration in the epithelial repair process exhibits genetic susceptibility and so appears analogous to abnormal mucosal phenotypes in asthma and other hypersecretory diseases. These concepts can be integrated into a scheme that incorporates epithelial, viral, and allergen components (designated an Epi-Vir-All paradigm) for a more complete explanation of the pathogenesis of airway disease. By extension, the same epithelial network is a target for therapy in airway disease and for improving host defense against respiratory viruses. Smart strategies have already been defined for reversing viral mimicry and engineering dominant-negative mutations that alter epithelial behavior, but full utilization of these approaches will depend on also achieving selective and high-level gene expression in the epithelium. This goal depends on turn on determining the critical biology of the airway epithelium in antiviral defense and airway disease and in particular on defining the contributions of specific viral and host genetic components.

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REFERENCES


KOYLA, TSUHYI S, BERTRAND C, HUANG S, AGUST M, ALAKA SS, and ANSHER N. Mice lacking the IFN-γ receptor have an impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. J Immunol 156: 2938–2955, 1996.


85. MARTIN RJ and GREEN MR. Transcriptional activation by viral immediate-early proteins: variations on a common theme. In: Transcriptional Regulation, edited by McKnight SL and Yamamoto KR.


