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A New Role for CXCL4 in Respiratory Syncytial Virus Disease

Although the impact of coronavirus disease (COVID-19) in future years is hard to predict, it seems certain that the ongoing morbidity and mortality caused by respiratory syncytial virus (RSV) (1, 2) is set to continue. Although many RSV vaccines are in advanced development (3), none have yet succeeded. Until there is a good vaccine or a specific treatment, the healthcare impact of RSV (4) and other respiratory viruses is set to continue unabated.

In this issue of the *Journal*, Han and colleagues (pp. 717–729) describe a hitherto unknown inhibitory effect of CXCL4 (previously known as platelet factor 4) on RSV infection, replication, and disease (5). The authors screened a complementary DNA library of human genes to identify factors that promote or inhibit transcription of RSV nucleoprotein in HeLa cells. They identified 122 proviral and 233 antiviral genes, of which 9 and 49, respectively, were of special interest to the authors. This screen reassuringly identified several known restriction factors (e.g., *IFNA1*, *IFNG*, and *IRF3*) but delivered the unexpected finding that CXCL4 was among the factors showing the greatest antiviral effect.

They confirmed this by overexpressing CXCL4 in culture, showing that RSV protein expression after infection was inhibited by CXCL4 and that only the secreted form of CXCL4 was effective. They went on to show that CXCL4 also inhibited replication of EV71 and HSV1 but not influenza A virus. In BALB/c mice, they showed that intranasal administration of CXCL4 prior to RSV challenge inhibited viral replication and lung inflammation but again had no effect on influenza. Taking samples from RSV-infected pediatric patients, they showed that CXCL4 in plasma, nasopharyngeal aspirate, and BAL fluid were increased after RSV infection. The airway CXCL4 level correlated positively with viral load and disease severity, but the increase in plasma levels was paradoxically negatively associated with more severe disease.

These are very interesting and novel data. CXCL4 has been previously shown to offer some protection against influenza and HIV (6, 7), but to our knowledge this is the first time it has been shown to have a protective effect against RSV infection and disease severity. The authors demonstrate that CXCL4 protects against RSV infection through inhibition of RSV attachment to heparan sulfate (HS) on the surface of target cells. This attachment step is considered to largely be mediated by the RSV attachment glycoprotein, although the RSV fusion (F) protein can also interact with HS (8). Antibodies can prevent the attachment of RSV to target cells; such neutralizing antibodies commonly target the F protein and are most effective when specifically directed against the pre-F conformation (9).

Directing antibodies against pre-F through vaccination has proved challenging, although exciting progress is being made (10). An alternative approach may be to increase resistance to RSV binding sites via HS, as demonstrated here with studies of CXCL4. Indeed, families of small-molecule antagonists of the HS–RSV interaction have been reported (11). Presumably, such approaches will be most effective prophylactically; clinical studies would be needed to determine the optimal time of intervention and to discover undesirable off-target effects.

Historically, CXCL4 was described as a secreted chemokine produced by platelets (leading to the synonym "platelet factor 4"), and the lung is an important source of platelets (12). This does not imply that the same is true of the nasal mucosa, but Han and colleagues' demonstration of elevated CXCL4 levels in nasal samples from severe cases of pediatric RSV bronchiolitis should direct attention to the study of megakaryocytes and platelets at other mucosal sites. Release of CXCL4 by platelets and associated cells might act to recruit monocytes and neutrophils to the airway (13), which is characteristically seen in severe RSV bronchiolitis. The elevation of CXCL4 in severe RSV bronchiolitis reported by Han and colleagues may indicate that the positive effects of suppression of viral replication are offset by the chemotactic recruitment of neutrophils, which may contribute to pathogenesis. Other platelet-derived chemokines such as CCL5 (C-C chemokine ligand 5) are also elevated during RSV infection (14). Despite this potential function in the early innate respiratory immune response,

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the role of platelets and of platelet-derived chemokines during respiratory infections is currently an understudied area.

The novel findings of Han and colleagues provide intriguing early insights into the possible roles of CXCL4 in RSV bronchiolitis and open new and tantalizing avenues of research. Additionally, the initial gain-of-function experiments identified a further 48 host factors that exhibited suppression of RSV replication. Powerful techniques such as those deployed by Han and colleagues are set to reveal the role of other host factors and to provide additional insights to the biology of viral infections and potential therapeutic strategies. ■

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Defining Airflow Obstruction: More Data, Further Clarity

Journals do not normally write editorials about letters. Of course, some letters merit attention being drawn to them. When Watson and Crick wrote a letter to *Nature* in 1953 proposing a new structure for deoxyribose nucleic acid (1), they changed the world and, ultimately, clinical practice. Their letter was really a scientific paper rather than normal correspondence, but research letters can make important contributions to our understanding, which is why this Journal accepts them. A good example of this process is the letter in this issue of the *Journal* by Neder and colleagues (pp. 760–762), which contributes to the continuing debate about how to interpret the FEV_1/FVC ratio when diagnosing chronic obstructive pulmonary disease (COPD) (2). Before considering their findings, it is worthwhile remembering how this debate began and why it is more important than simple semantics.

Since the 1950s, when the FEV_1/FVC ratio was first described, respiratory physiologists have empirically used a value of 0.7 to define airflow obstruction, as it related well to other more invasive physiological measurements of airflow limitation in patients. This convention was followed when the Global Initiative in Chronic Obstructive Lung Disease incorporated spirometry into their definition of COPD and has remained so since (3).There was a general awareness that the ratio declined with age in apparently healthy people, a point highlighted in a study of older Norwegians (4), which was one of the first to point out the implications of this change for the diagnosis of COPD. Subsequently, the Global Lung Function Initiative developed values for the lower limit of normal (LLN) of the ratio, which meant that many older people with ratios below 0.7 would be considered healthy, and, if

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