by increasing the binding of HES1 to the *Klf4* promoter¹. Subsequent transcription profiling revealed that the intestines of mice treated with the inhibitor and dexamethasone expressed more *Ccnd2*, a cell cycle regulator, than did those of mice treated with the inhibitor alone. The authors provide evidence that the dexamethasone-induced expression of *Ccnd2*, at least in part, protects the mice from inhibitor-induced goblet cell metaplasia, although its observed effect on *Klf4* downregulation is indirect¹.

The known toxicity of dexamethasone treatment includes osteopenia, hypertension and muscle atrophy; however, the potential effects of long-term inhibition of γ -secretase remain unknown. The enzyme γ -secretase targets more than 30 physiologically important transmembrane proteins, including the amyloid precursor protein involved in Alzheimer's disease¹¹. Given that there are at least six different γ -secretase complexes in humans, subtype-specific inhibitors might be developed that have less broad side effects¹².

Clinical trials of γ -secretase inhibitors have been hindered by the considerable gastrointestinal problems experienced by the participants¹². Therefore, it will be necessary to determine

whether the combination therapy with dexamethasone is as safe in humans as it seems to be in mice. No doubt it will take time to determine the optimal safe dosage for treatment; Real *et al.* report that in a small number of their mice, the combined treatment had to be stopped owing to excessive weight loss¹. The combination treatment also increased lymphoid atrophy in the thymus and spleen, thereby potentially diminishing normal immunity.

Despite the promise of this approach for NOTCH1-activated T-ALL, not all people with this condition would be expected to respond. Eight percent of T-ALL samples harbor mutations in or show homozygous deletion of *FBW7*, a gene that encodes a ubiquitin ligase responsible for NOTCH1 ICN1 turnover. T-ALL cell lines carrying such mutations are resistant to treatment with γ -secretase inhibitors¹³.

The findings of Real *et al.*¹ may have broader implications, as NOTCH1 signaling is involved in many cancers¹⁴. By inference, the combination therapy might also be beneficial for solid tumors with aberrant NOTCH1 signaling. Likewise, γ -secretase inhibitors inhibit the production of amyloidogenic β -amyloid peptides involved in Alzheimer's disease, and this

work may provide new ways for treating that condition.

The new findings will initiate a flurry of research activity to further validate the combination therapy in additional preclinical studies¹. Provided that the outcome will be positive, the approach will be ready to move into phase 1 trials.

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Fixing a failed vaccine

Steven M Varga

A trial of a childhood vaccine against a common respiratory virus went terribly wrong in the early 1960s. Instead of protecting children, the vaccine exacerbated disease in response to infection. We now have a better understanding as to why (pages 34–41).

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection in infants. By two years of age, more than 90% of children have been infected at least once with this virus. RSV infections typically cause mild illness; however, severe respiratory disease can occur and is associated with symptoms such as bronchiolitis and wheezing. RSV infection is also common in the elderly, in whom it can be deadly. Despite the enormous impact of RSV on human health, the design of a safe and successful vaccine has eluded researchers.

During the 1960s, researchers thought they had a vaccine. But they were wrong—the

experimental, formalin-inactivated RSV vaccine administered to children not only failed protect them but also exacerbated their illness in response to natural RSV exposure. Many of the children developed bronchiolitis and pneumonia so severe that it required hospitalization, and, unfortunately, two of the vaccinated children died¹.

The formalin-inactivated RSV vaccine did elicit antibodies—but these were not neutralizing, meaning that they did not inactivate the virus. High titers of RSV were recovered from the lungs of the two children that died, demonstrating the ineffectiveness of the vaccine in eliciting a protective immune response¹.

In this issue of *Nature Medicine*, Delgado *et al.*² provide insight into the failure of the formalin-inactivated RSV vaccine. They report that inactivated forms of RSV do not sufficiently activate various pattern recognition receptors, molecules important for the

recognition of pathogens by immune cells and expressed by several cell types including B cells, the cells that produce antibodies. As a result of this poor activation, B cells produce only low-avidity antibodies that poorly recognize the virus. Because the vaccine elicited such poor antibodies, it failed to inhibit virus replication and resulted in enhanced disease, probably caused by the host immune response.

The vaccine-enhanced disease exhibited by the children during the 1960s vaccine trial can be recapitulated in mice. Early studies in the mouse model clearly established that the host immune response contributes to the development of RSV vaccine-enhanced disease. As in people, immunization with formalin-inactivated RSV results in the production of non-neutralizing RSV-specific antibodies³. After RSV challenge, these non-neutralizing antibodies cause the formation of immune

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Figure 1 Differential activation of pattern recognition receptors by live RSV versus formalin-inactivated RSV. The F protein of RSV is capable of being recognized by TLR4 expressed on the cell surface of host cells. RSV typically enters the host cell through fusion at the plasma membrane. In some cases, virus entry can occur via receptor-mediated endocytosis of RSV virions coated with antibodies. Once inside the cell, a number of host cell pattern recognition receptors are capable of recognizing virus-derived nucleic acids. RIG-I and MDA-5 can recognize double-stranded RNA (dsRNA) intermediates, whereas TLR7 is activated by virus-derived single-stranded RNA (ssRNA). The combined stimulation of TLR4, TLR7, RIG-I and/or MDA-5 by live RSV infection leads to the activation of multiple downstream signaling pathways that ultimately results in full activation of the B cell and is necessary to achieve antibody affinity maturation. Delgado *et al.*² demonstrate that formalin-inactivated RSV fails to engage and activate pattern recognition receptors found inside the host cell, such as TLR7, RIG-I and MDA-5, because of the failure of the virus to replicate. Therefore, only TLR4 is sufficiently stimulated after formalin-inactivated RSV vaccination. The B cells fail to produce high-avidity antibodies in the absence of sufficient TLR stimulation.

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complexes consisting of antibodies bound to the surface of the RSV virion⁴.

Previous work from this group showed that complement components activated by these immune complexes were necessary for bronchoconstriction during RSV infection of mice previously immunized with the vaccine⁵. What has been unclear is why immunization results in the production of these nonprotective antibodies, impeding the development of new RSV vaccines for decades.

One hypothesis is that formalin treatment disrupts the folding of proteins on the surface of the RSV virion that harbors protective antibody epitopes—accounting for the inability to induce a neutralizing antibody response^{3,6,7}. The results obtained by Delgado *et al.*² suggest that the situation is a bit more complicated.

Delgado *et al.*² compared the antibody response after immunization with either live RSV or inactivated forms of the virus. As expected, inactivated forms of the virus elicited only low-avidity antibodies, whereas live RSV induced neutralizing antibodies of high avidity. Immunization with the inactivated forms of RSV resulted in increased airway hyperresponsiveness and increased virus titers in the lung after RSV challenge as compared to mice previously vaccinated with live virus.

A number of Toll-like receptors (TLRs) and other pattern recognition receptors are

activated by RSV infection. The fusion protein of RSV is recognized by TLR4 on the cell surface⁸, the RSV single-stranded RNA genome is recognized by TLR7 (ref. 9) and RSV double-stranded RNA is recognized by the helicases retinoic acid-inducible gene-1 (RIG-I) and melanoma differentiation-associated protein-5 (MDA-5)¹⁰ expressed inside of host cells (Fig. 1). Delgado *et al.*² hypothesized that the inability of inactivated forms of RSV to replicate compromised their ability to activate pattern recognition receptors, resulting in less efficient B cell stimulation.

They initially tested this by examining mice deficient in myeloid differentiation factor-88, an adaptor molecule used by several pattern recognition receptors to mediate signals². They showed that RSV infection of myeloid differentiation factor-88-deficient mice resulted in the production of lower avidity antibodies specific to RSV². Finally, they found that stimulation of TLRs increased the avidity of the antibodies elicited after immunization with inactivated forms of RSV². Mice treated this way had lower airway hyperresponsiveness and lower virus titers in the lung after RSV challenge infection.

These findings implicate low-avidity antibodies in the development of RSV vaccine-enhanced disease. But there are probably other events that also contribute to disease. Previous work has shown that CD4⁺ T cells are required to induce pneumonia after RSV

challenge of mice previously immunized with formalin-inactivated RSV¹¹. Moreover, recent research has indicated that formalin-inactivated RSV fails to elicit a RSV-specific CD8⁺ T cell response¹².

Taken together, these studies indicate that immunization with formalin-inactivated RSV elicits a low-avidity, RSV-specific antibody response, a robust RSV-specific CD4⁺ T cell response and no cytotoxic CD8⁺ T cell response. The exacerbated disease in the children was probably due to these unique circumstances that together created the perfect storm. A strong, RSV-specific CD4⁺ T cell response would have been maintained for a longer time in the face of increased virus titers, owing to the absence of an effective cytotoxic CD8⁺ T cell response and an ineffective antibody response.

The findings could have broader implications for vaccine development, as inappropriate immune responses elicited by vaccination are not limited to RSV. For instance, immunization with an experimental formalin-inactivated measles virus vaccine resulted in an increased incidence of atypical measles infections among the vaccinees¹³. A number of effective formalin-inactivated virus vaccines are currently in use, suggesting that the relative balance between a protective versus pathogenic immune response elicited by vaccination with a formalin-inactivated vaccine is dependent on the nature of the protective immunity required for a particular pathogen.

Future studies aimed at dissecting out the distinct roles of antibodies and T cells in mediating RSV vaccine-enhanced disease are essential for gaining a further understanding of the failure of the formalin-inactivated RSV vaccine. The intriguing results obtained by Delgado *et al.*² suggest that the addition of TLR-stimulating adjuvants may conceivably be enough to make a safe and effective vaccine, but we need to know more before such a treatment can become a reality.

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