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INFLUENZA VIRUS

Lethal weapons

Certain strains of influenza virus can cause a lethal infection even in young healthy adults. However, the relative contribution of the virus versus the host immune system to driving tissue damage and lethality remains unresolved. Using a systems approach in mice, Brandes, Germain and colleagues now show that it is the unrestrained innate inflammatory response of the host, driven by a feedforward circuit involving pro-inflammatory neutrophils, that underlies at least some forms of influenza-induced lethality.

In this study, mice were infected with the non-lethal H1N1 virus A/Texas/36/91 (Tx91), or sublethal or lethal doses of the highly pathogenic H1N1 virus A/Puerto Rico/8/34 (PR8). Gene expression in lung samples from each group of mice was then assessed by microarray analysis at various time points post infection. Using these microarrays, the authors generated 50 modules of gene sets with coordinated patterns of regulation during influenza virus infection.

Further analyses of these modules identified patterns of increased gene expression early after infection (days 1 to 3) that were associated with innate inflammatory signatures. One of these signatures represented genes involved in the antiviral immune response, such as those encoding type I interferons (IFNs), IFNγ and IFN-regulatory factors, and another represented inflammatory signalling cascade genes. However, only the signature of genes involved in the inflammatory signalling cascade was closely associated with fatal disease. The gene sets in this signature contained genes associated with neutrophil recruitment, myeloid cell differentiation, pro-inflammatory cytokine production and endothelial cell activation.

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Next, five different cell populations were purified from the lungs of mice 48 hours post infection. Flow cytometric analyses confirmed previous observations that the numbers of neutrophils and inflammatory monocytes are greatly increased in the lungs of influenza virus-infected mice, particularly in those infected with PR8. Repetition of the above transcriptional analysis in the isolated cell populations showed that the antiviral signature was not restricted to the infected cell population (that is, non-haematopoietic cells) but involved all of the cell types that were analysed. By contrast, the inflammatory signalling signature associated with lethality seemed to mostly originate from neutrophils in the infected lungs. Interestingly, many of the genes in this signature were also expressed by mature neutrophils isolated from uninfected mice. This suggests that both preformed and newly synthesized factors drive the lethal signature.

Given that early neutrophil infiltration seems to be a characteristic of lethal, but not non-lethal, influenza virus infection, an important next step was to understand how neutrophil infiltration during lethal infection is controlled. Neutrophils from uninfected mice were found to constitutively express mRNA for the neutrophil chemoattractant CXC-chemokine ligand 2, and in mice infected with lethal doses of PR8, more neutrophil-attracting chemokines — mostly originating from the neutrophils themselves — were produced. This suggests that there is a feedforward mechanism of neutrophil recruitment that depends on neutrophil activation during influenza virus infection.

Using automated imaging analysis, PR8 was shown to spread further through the tissue than Tx91. Furthermore, much higher levels of viral proteins (which can function as pathogen-associated molecular patterns (PAMPs)) were detected in the lungs of PR8-infected mice compared with Tx91-infected mice. These and other data support the hypothesis that the spread of the PR8 virus contributes to excess encounters of neutrophils with PAMPs, which drives a feedforward circuit of neutrophil influx and activation by overcoming the elevated activation threshold of these myeloid cells. This results in uncontrolled tissue damage and ultimately in death. Indeed, reducing (but not eliminating) neutrophil numbers in PR8-infected mice improved survival by reducing the damaging effects of excessive neutrophil activation.

Thus, targeting this feedforward circuit might represent a therapeutic strategy for the treatment of infection by certain influenza virus strains.

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ORIGINAL RESEARCH PAPER Brandes, M. *et al.* A systems analysis identifies a feedforward inflammatory circuit leading to lethal influenza infection. *Cell* **154**, 197–212 (2013)