Role of type 2 deiodinase in response to acute lung injury (ALI) in mice

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AUTHOR SUMMARY

Triiodothyronine 3 (T\textsubscript{3}), the active form of thyroid hormone, plays an essential role in maintaining normal physiology. In healthy people, T\textsubscript{3} is generated when an enzyme known as type 2 iodothyronine deiodinase (D2) converts a less active form of thyroid hormone (thyroxine, T\textsubscript{4}) to T\textsubscript{3}. However, in acute illness—specifically lung injury—the circulating concentration of the active T\textsubscript{3} is reduced, and T\textsubscript{4} is converted to a biologically inactive form known as “reverse T\textsubscript{3}” (rT\textsubscript{3}) by type 3 deiodinase (D3). In this way, deiodinase is a regulator of thyroid hormone metabolism (1).

Although this topic has been the subject of much research, it remains unclear whether tissues are functionally hypothyroid (i.e., deficient in thyroid hormone) during severe illness, as reflected by low levels of thyroid hormone in serum and tissue (2, 3). An additional conundrum in the treatment of patients with severe illness is whether the change in thyroid hormone concentrations is protective or detrimental at the tissue level and whether treatment with T\textsubscript{3} could overcome the inflammatory response to the injury. In this study, we explored these unresolved questions in greater detail.

Our findings show that inflammatory markers and D2 levels in the lung are stimulated in a mouse model of lung injury (4). Indeed, thyroid hormone concentrations in the blood reproduce nonthyroidal illness, a condition in which systemic levels of circulating thyroid hormone levels are dramatically lowered in response to ventilatory-induced lung injury (VILI). Furthermore, by studying engineered mice that lack D2, we found that increased D2 expression compensated for the lower levels of T\textsubscript{3} available to tissue subjected to VILI. Finally, we demonstrated that systemic T\textsubscript{3} treatment can reverse the inflammatory markers of injury.

We performed immunohistochemistry, a form of tissue analysis that relies on antibodies to identify the lung cells in which D2 is found. In wild-type and normal lungs of spontaneously breathing (nonventilated) control mice we found that D2 was present in the airway epithelium and in the pulmonary endothelium. VILI-challenged wild-type mice demonstrated markedly increased levels of D2 protein in the pulmonary endothelium and epithelium compared with spontaneously breathing wild-type mice; however, D2 immunoreactivity was not detected in inflammatory cells.

To determine whether the D2 induction of VILI-challenged wild-type mice had a protective or detrimental role in inflammatory lung injury, we exposed mice with a targeted D2 deletion (D2KO mice) to VILI. Both VILI-challenged wild-type and D2KO mice exhibited an increase in lung vascular permeability compared with spontaneously breathing animals. In addition, D2KO mice had a slightly greater, although not statistically significant, increase in bronchoalveolar lavage protein content following VILI compared with wild-type mice. Further, both wild-type and D2KO animals showed increased infiltration of inflammatory white blood cells in the walls of the alveoli, the small air sacs within the lungs, and had increased hyaline membrane formation after exposure to VILI. These responses were more dramatic in D2KO mice than in wild-type animals, indicating that the absence of D2 was harmful and did not protect the lungs. Cytokines and chemokines are secreted signaling molecules that often help modulate the immune system.

After VILI exposure, D2KO mice displayed marked elevations of several lung cytokines and chemokines. These results suggest that D2KO mice are more susceptible than wild-type mice to VILI, therefore indicating that D2 is protective against VILI. To identify additional transcriptional pathways responsible for the D2KO lung phenotype, we performed a microarray analysis of lungs from spontaneously breathing wild-type and D2KO mice as well as lungs from wild-type and D2KO mice after VILI. This approach identified additional genes with reported roles in the regulation of inflammation and damage.


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including genes encoding heat-shock protein 1 (Hsp1), Serpina 3G, matrix metallopeptidase 9 (Mmp9), and resistin-like γ (Retnlγ). We found that each of these genes was down-regulated or not expressed in VILI-exposed D2KO mice, indicating that the absence of D2 modifies the lung transcriptome following exposure to VILI. Consequently, these changes may increase the inflammatory response and decrease cytoprotective defenses against VILI.

We then pretreated D2KO mice with triiodo-l-thyronine before exposure to VILI to determine whether a potential lung thyroid hormone deficiency accounts for the increased susceptibility of D2KO mice to inflammatory lung injury. Our findings revealed that D2KO mice are more susceptible than wild-type mice to VILI; however, we did not detect differences in serum thyroid hormone concentrations between wild-type and D2KO mice. To investigate whether thyroid hormone deprivation in the lung following exposure to VILI reproduced the findings in the D2KO mice, hypothyroid mice were subjected to VILI. Induction of hypothyroidism was confirmed by low or undetectable levels of serum T₃, T₄, and rT₃ and high levels of thyroid-stimulating hormone.

The lungs of hypothyroid spontaneously breathing mice had higher protein levels in bronchoalveolar lavage than the lungs of euthyroid (normal) spontaneously breathing mice. Similar trends were found in hypothyroid VILI mice, but the differences did not reach statistical significance. Hypothyroid VILI-treated wild-type mice had marked elevations in lung chemokines and cytokines compared with euthyroid animals, indicating that hypothyroid mice had increased susceptibility to VILI.

Hypothyroidism, as expected, increased D2 enzymatic activity. Curiously, however, in contrast to the results obtained for euthyroid animals, exposure to VILI induced neither D2 mRNA nor enzymatic activity in hypothyroid animals. Our results show that VILI up-regulates the expression of D2 and enzymatic activity in the lung. Furthermore, we demonstrated that D2 up-regulation likely plays a protective role during lung injury. Therefore, induction of D2 in response to VILI may represent an adaptive response to circulating low T₃ levels by generating more local T₃. Alternatively, D2 could be up-regulated in response to the local injury as part of an inflammatory pathway involving NF-κB, an important mediator of immune and inflammatory responses that has been strongly implicated in acute lung injury. Evidence supports a role for NF-κB in inducing transcription of human and rat D2 gene expression, and, indeed, a potent NF-κB–binding site has been demonstrated in the human D2 gene (5). The induction of D2 expression and especially an increase in the protein and its activity after mechanical ventilation constitute a protective mechanism against VILI, likely by compensating for the local reduction of thyroid hormone signaling, which may dampen the inflammatory response to VILI (Fig. P1). Our findings support a role for D2 in the regulation of the cellular response to acute lung injury and suggest that T3 could prove an effective treatment in such situations.