News Release

Title

CD4$^+$ T cells are essential for the development of destructive thyroiditis induced by anti-PD-1 antibody in thyroglobulin-immunized mice

Key Points

- Immune checkpoint inhibitors (ICI) have been widely used as a promising treatment in patients with advanced malignancies, however, those drugs also cause immune-related adverse events (irAEs).
- Among endocrine irAEs, although thyroid dysfunction has been reported to be the most frequently observed side effect during treatment with anti-programmed cell death-1 antibodies (PD-1-Ab), the mechanism underlying development of thyroid-irAE remains unclear. In this study, we established a mouse model of thyroid-irAE, which was induced by PD-1-Ab injections.
- PD-1-Ab administration 2.5 months after immunization with thyroglobulin (Tg) caused destructive thyroiditis in CBA/J mice. In contrast, mice injected with PD-1-Ab without prior immunization with Tg did not develop destructive thyroiditis.
- The frequencies of central and effector memory CD4$^+$ T cell subsets were increased in the cervical lymph nodes of mice with destructive thyroiditis induced by PD-1-Ab. Histopathological analysis revealed infiltration of CD4$^+$ T cells expressing a cytotoxic molecule, granzyme B, in the thyroid glands.
- To clarify which cells were directly involved in the development of destructive thyroiditis induced by PD-1-Ab, CD4$^+$ T cells, CD8$^+$ T cells or CD20$^+$ B cells were depleted before the first administration of PD-1-Ab. The development of destructive thyroiditis induced by PD-1-Ab was completely prevented by the prior depletion of CD4$^+$ T cells.
- The frequencies of central and effector memory CD4$^+$ T cells expressing the cytotoxic marker CD27 were higher in peripheral blood mononuclear cells collected from patients with thyroid-irAE induced by PD-1-Ab versus those without.
- These data suggest cytotoxic CD4$^+$ T cells are essential for the development of destructive thyroiditis induced by anti-PD-1 antibody.

Summary

Immune checkpoint inhibitors (ICI) have been used as a promising treatment in patients with advanced malignancies. However, those drugs also cause immune-related adverse events (irAEs), possibly due to the activation of an autoimmune response against tissues, including lung, liver, skin, colon and endocrine glands. Among endocrine irAEs, thyroid dysfunction has been reported to be the most frequently observed side effect during treatment with anti-programmed cell death-1 antibodies (PD-1-Ab) and causes transient thyrotoxicosis.

Although infiltrating cytotoxic $CD8^+$ T cells have been observed in some tumors isolated from patients treated with PD-1-Ab, the mechanisms underlying development of irAE including thyroid-irAE remain unclear. In this study, we established a mouse model of destructive thyroiditis induced by PD-1-Ab to clarify the mechanism underlying the development of this disease and analyzed which T cell subsets have a critical role in the development of the disease. PD-1-Ab administration 2.5 months after immunization with thyroglobulin (Tg) which is protein specific for the thyroid gland caused destructive thyroiditis. In this mouse model, $CD4^+$ T cells, among which the frequencies of central and effector memory subsets were increased by PD-1-Ab injections, showed cytotoxic characteristics and Tg specificity. In addition, depletion of $CD4^+$ T cells prior to PD-1-Ab administration completely prevented the development of destructive thyroiditis induced by PD-1-Ab, whereas depletion of $CD8^+$ T cells only partially prevented the development of thyroiditis. Furthermore, adoptive transfer of $CD4^+$ T cells from mice developing destructive thyroiditis induced by PD-1-Ab caused destruction of thyroid follicular architecture in the irradiated recipient mice. Finally, the frequencies of cytotoxic $CD4^+$ T cells were higher in peripheral blood mononuclear cells collected from patients with thyroid-irAE induced by PD-1-Ab versus those without. These data suggest a critical role for cytotoxic memory $CD4^+$ T cells activated by PD-1-Ab in the pathogenesis of thyroid-irAE. These findings have implications for understanding the pathogenesis of irAEs induced by PD-1-Ab and for establishing preventive strategies against adverse events associated with ICI treatments.

## Research Background

Immune checkpoint inhibitors (ICI) have been used as a promising treatment in patients with advanced malignancies. However, those drugs also cause immune-related adverse events (irAEs), possibly due to the activation of an autoimmune response against tissues, including lung, liver, skin, colon and endocrine glands. Among endocrine irAEs, thyroid dysfunction has been reported to be the most frequently observed side effect during treatment with anti-programmed cell death-1 antibodies (PD-1-Ab). The mechanism underlying the development of this disease remains unclear.

## Research Results

In this study, PD-1-Ab administration 2.5 months after immunization with thyroglobulin (Tg), a protein specifically expressed in thyroid gland, caused destructive thyroiditis. PD-1 was predominantly expressed on $CD4^+$ T cells. PD-L1, a ligand of PD-1, was expressed on antigen presenting cells such as macrophages in the thyroid glands and cervical lymph nodes of this mouse model. Furthermore, $CD4^+$ T cells, among which the frequencies of central and effector memory subsets were increased by PD-1-Ab injections, showed cytotoxic characteristics and Tg specificity in this mouse model. Next, in order to investigate which cells were directly involved in the development of destructive thyroiditis induced by PD-1-Ab, $CD4^+$ T cells, $CD8^+$ T cells or $CD20^+$ B cells were depleted before the first administration of PD-1-Ab. Depletion of $CD4^+$ T

cells prior to PD-1-Ab administration completely prevented the development of destructive thyroiditis induced by PD-1-Ab, whereas depletion of $CD8^+$ T cells only partially prevented the development of thyroiditis. Furthermore, adoptive transfer of $CD4^+$ T cells from mice developing destructive thyroiditis induced by PD-1-Ab caused destruction of thyroid follicular architecture in the irradiated recipient mice. Next, peripheral blood mononuclear cells (PBMCs) from the patients who developed destructive thyroiditis induced by PD-1-Ab, nivolumab or pembrolizumab (n = 6) and patients who did not develop any irAEs (n = 7) were analyzed. The frequencies of cytotoxic $CD4^+$ T cells were higher in PBMCs collected from patients with thyroid-irAE induced by PD-1-Ab versus those without. These findings suggest that cytotoxic $CD4^+$ T cells in memory cell subsets activated by PD-1-Ab directly damaged thyroid glands and had an essential role in the development of thyroid-irAE. These findings have implications for understanding the pathogenesis of irAEs induced by PD-1-Ab and for establishing preventive strategies against adverse events associated with ICI treatments.

## Research Summary and Future Perspective

This study shows a critical role for cytotoxic memory $CD4^+$ T cells activated by PD-1-Ab in the pathogenesis of thyroid-irAE, although $CD8^+$ T cells are generally considered to have an important role in cancer immunotherapy using ICI. These findings are expected to lead to the understanding of the pathogenesis of irAEs induced by PD-1-Ab and the establishment of novel preventive strategies against adverse events associated with ICI treatments.

## Publication

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DOI：10.1126/scitranslmed.abb7495

Japanese ver：
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