Identification of RORg$^+$T Cells as Key Players in Thyroid Autoimmunity From Checkpoint Immunotherapy

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Purpose: Immune checkpoint inhibitors (ICI) are powerful new cancer therapies that leverage the body's own immune system to attack cancer cells. Unfortunately, their use may be limited by the development of immune-related adverse events (IrAE) in up to 60% of patients. Thyroiditis is a common IrAE, with shared and distinct features from spontaneous thyroid autoimmunity, *i.e.* Hashimoto thyroiditis (HT). The cause of IrAE remains unknown, however, recent data suggest that toxicity can be uncoupled from anti-tumor effects.

Methods: We developed a novel mouse model to study mechanisms of IrAE, in which ICI (anti-PD-1 and/or anti-CTLA-4) treatment leads to multi-organ immune infiltrates, including thyroiditis. To understand immune changes occurring with ICI-autoimmunity, we first evaluated changes in the frequency and activation status of different immune cells in our mice using immunohistochemistry (IHC) and flow cytometry. Then we confirmed these findings in peripheral blood and thyroid fine needle aspiration (FNA) specimens from patients with ICI-thyroiditis, HT, or no IrAE, using flow cytometry and single cell RNA sequencing (scRNAseq) techniques.

Results: In our mouse model, ICI treatment of autoimmune-prone non-obese diabetic mice induces multi-organ autoimmunity. Modeling ICI-IrAE observed in humans, our mice developed increased immune infiltrates in multiple tissues (*e.g.* thyroid, colon, liver, lung), autoantibodies, and acceleration of underlying autoimmune risk (*i.e.* diabetes). Increased frequency of autoimmune disease was seen with combination (anti-PD-1 + anti-CTLA-4) *vs.* single agent ICI. We found increased IL-17A$^+$ T cells in secondary lymphoid tissues of ICI-treated mice, a cytokine produced by RORγ^+ Th17 and Tc17 cells and associated with autoimmunity. IHC studies on thyroid infiltrates showed accumulation of CD4$^+$ and CD8$^+$ T cells and macrophages in ICI-treated *vs.* isotype control mice. This finding was confirmed by flow cytometry analyses of thyroid-infiltrating leukocytes in ICI-thyroiditis mice, which showed significantly increased T cells, specifically RORγ^+ T cells, and rare B220$^+$ B, CD11b$^+$ myeloid, or NKp46$^+$ NK cells. In patients with ICI-thyroiditis, thyroid FNA showed that thyroid immune infiltrates were predominately T cells. scRNAseq studies in patients with ICI-thyroiditis showed enrichment of Th17 and Tc17 (RORγ^+ IL23R$^+$ CD161$^+$) T cells, compared to ICI-treated patients without IrAE.

Conclusion: We have identified a role for RORγ^+ Th17 and Tc17 cells in thyroid autoimmunity from ICI using a newly developed mouse model of ICI-associated IrAE and translational studies in patients with ICI-thyroiditis. Th17 and Tc17 cells have previously been associated with spontaneous autoimmune disease, including HT, but have not yet been characterized in IrAE. These cells provide a potential therapeutic target for prevention of endocrine IrAE from ICI.

Thyroid

THYROID AUTOIMMUNITY, COVID-19 & THYROID DISEASE

Insights From Prospective Follow-up of Thyroid Function and Autoimmunity Among Covid-19 Survivors

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Objective: Occurrence of Graves' disease and Hashimoto's thyroiditis after coronavirus disease 2019 (COVID-19) raised the concern about severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggering thyroid autoimmunity. Uncertainties remain regarding incident thyroid dysfunction and autoimmunity among COVID-19 survivors. We carried out a prospective study to characterize the evolution of thyroid function and autoimmunity among COVID-19 survivors. **Method:** Consecutive adult patients, without known thyroid disorders, admitted to Queen Mary Hospital for confirmed COVID-19 from 21 July to 21 September 2020 were included. Serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3) and anti-thyroid antibodies were measured on admission and at 3 months. Positive anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) was defined by >100 units. **Results:** Among 200 COVID-19 survivors, 122 had reassessment thyroid function tests (TFTs) (median age: 57.5 years; 49.2% men). Baseline characteristics of patients who did and did not have reassessment were comparable. Among the 20 patients with baseline abnormal TFTs on admission, mostly low fT3, 15 recovered. Of the 102 patients with normal TFTs on admission, two (2.0%) had new onset abnormal TFTs, which may represent TFTs in different phases of thyroiditis (one had mildly elevated TSH 5.8 mIU/L, with normal fT4 [16 pmol/L] and fT3 [4.3 pmol/L], the other had mildly raised fT4 25 pmol/L with normal TSH [1.1 mIU/L] and fT3 [4.7 pmol/L]). Among 104 patients with anti-thyroid antibody titers reassessed, we observed increases in anti-TPO (baseline: 28.3 units [IQR 14.0-67.4] vs reassessment: 35.0 units [IQR: 18.8-99.0]; p<0.001) and anti-Tg titers (baseline: 6.6 units [IQR 4.9-15.6] vs reassessment: 8.7 units [IQR: 6.6-15.4]; p<0.001), but no change in anti-TSHR titer (baseline: 1.0 IU/L [IQR: 0.8-1.2] vs reassessment: 1.0 IU/L [IQR: 0.8-1.3]; p=0.486). Of the 82 patients with negative anti-TPO at baseline, 16

had significant interval increase in anti-TPO titer by >12 units (2×6 [precision of the anti-TPO assay in normal range being 6 units per SD]), of these, four became anti-TPO positive. Factors associated a significant increase in anti-TPO titer included worse baseline clinical severity (p=0.018), elevated C-reactive protein during hospitalization (p=0.033), and higher baseline anti-TPO titer (p=0.005). **Conclusion:** Majority of thyroid dysfunction on admission recovered during convalescence. Abnormal TFTs suggestive of thyroiditis could occur during convalescence, though uncommon. Importantly, we provided the novel observation of an increase in anti-thyroid antibody titers post-COVID-19, suggesting the potential of SARS-CoV-2 in triggering thyroid autoimmunity, which warrants further follow-up for incident thyroid dysfunction among COVID-19 survivors.

Thyroid

THYROID AUTOIMMUNITY, COVID-19 & THYROID DISEASE

Management of Thyrotoxicosis Induced by PD1 or PD-L1 Blockade

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Context: Thyrotoxicosis is a common immune-related adverse event in patients treated with PD1 or PD-L1 checkpoint inhibitors. A detailed endocrinological assessment, including thyroid ultrasound and scintigraphy is missing, as are data on response to treatment and follow-up. **Objectives:** To better characterize the thyrotoxicosis secondary to immune checkpoint inhibitors, gaining insights into pathogenesis and informing management. **Methods:** We conducted a prospective cohort study of 20 consecutive patients who had normal thyroid function before starting immunotherapy and then experienced thyrotoxicosis upon PD1 or PD-L1 blockade. Clinical assessment was combined with thyroid ultrasound, scintigraphy, and longitudinal thyroid function tests. **Results:** Five patients had normal scintigraphic uptake (Sci+), no serum antibodies against the TSH receptor, and remained hyperthyroid throughout follow-up. The other 15 patients had no scintigraphic uptake (Sci-) and experienced destructive thyrotoxicosis followed by hypothyroidism (N= 9) or euthyroidism (N= 6). Hypothyroidism was more readily seen in those with normal thyroid volume than in those with goiter (P= 0.04). Among Sci- subjects, a larger thyroid volume was associated to a longer time to remission (P<0.05). Methimazole (MMI) was effective only in Sci+ subjects (P<0.05). **Conclusions:** Administration of PD1 or PD-L1 blocking antibodies may induce two different forms of thyrotoxicosis that appear similar in clinical severity at onset: a type 1 characterized by persistent hyperthyroidism that requires treatment with MMI, and a type 2 characterized by destructive and transient thyrotoxicosis that evolves to hypo- or eu-thyroidism. Thyroid scintigraphy and ultrasound help differentiating and managing these two forms of iatrogenic thyrotoxicosis

Thyroid

THYROID AUTOIMMUNITY, COVID-19 & THYROID DISEASE

Managing Thyroid Nodules in Qatar During the COVID19 Pandemic

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Background: The Coronavirus disease 2019 (COVID-19) pandemic impacted health care systems in all countries, including Qatar. Hamad Medical Corporation (HMC); In compliance with recommendations, suspended all non-urgent procedures, including thyroid fine needle aspiration biopsies (FNAB). Thyroid nodules are second most common cause of referral to HMC endocrine clinic. FNABs are gold standard to differentiate benign from malignant nodules.[1-2]
Methods: Our approach includes a teleconsultation to obtain patient's history and risk factors. Reviewing neck ultrasound (US), obtaining a calcitonin level if indicated, considering comorbidities associated with a high risk of COVID-19 morbidity and mortality.[3]
Results: We developed a pathway triaging thyroid (FNAB) to:1-Urgent: patients at higher risk of aggressive thyroid malignancy. Benefits of early detection and treatment outweigh the risk of COVID-19 exposure.[4] FNAB should not be delayed.2-Semi-urgent: patients at low risk for COVID-19 and high suspicion thyroid nodules, but no evidence that early detection improves survival[2], FNAB may be delayed up to 12 months.3-Non-urgent: patients with asymptomatic nodules that have low or intermediate suspicion US pattern.[2] Also, includes nodules with ATA high suspicion US pattern in pregnant women and patients at high risk for COVID-19. The risks outweigh the benefits. FNAB should be delayed until outbreak is controlled.[4] When urgent FNAB is indicated, safety of patients and medical staff needs to be addressed.[5] We recommend testing patient for COVID-19 before FNAB, utilizing US guidance with rapid on-site adequacy evaluation in all cases. Cervical lymph node FNAB with TG washout should be done if indicated. The patient should wear a mask. All medical staff involved should wear personal protective equipment (PPE). The operator should wear N95 mask and face shield. The