Spontaneous lymphocytic thyroiditis in interferon regulatory factor-1 deficient non-obese diabetic mice

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ABSTRACT. Interferon regulatory factor-1 (IRF-1) is a transcription factor involved in interferon-mediated immune reaction, CD8+ T cell differentiation and development of T helper 1 immune reaction. We have recently demonstrated that IRF-1 is pivotal in iodine-induced lymphocytic thyroiditis (LT) in non-obese diabetic (NOD) mice. However, it remains unclear whether the mechanism involved in spontaneous LT is identical with iodine-induced LT in NOD mice. To determine the role of IRF-1 in spontaneous LT, we used IRF-1 deficient NOD mice as well as IRF-1 +/- and +/- mice which were free from treatments for LT induction, and LT was evaluated at 24 weeks of age. IRF-1 +/-, +/- and --/-- mice developed LT spontaneously, and there were no differences among the 3 IRF-1 genotypes in the incidence and severity of LT. Whereas both CD4+ and CD8+ T cells were present in the diseased thyroid of IRF-1 +/- mice, CD8+ T cells were absent in the thyroid of IRF-1 --/-- mice. MHC class II antigen expression was induced in the inflamed thyroid of IRF-1 --/-- mice comparable to IRF-1 +/- mice. There was a selective reduction in the number of CD8+ T cells in the spleen of IRF-1 --/-- mice. IFNγ production, but not IL-10, by concanavalin A-stimulated splenocytes was significantly reduced in IRF-1 deficient mice. These results suggest that IRF-1 plays only a minor role in spontaneous LT in NOD mice and, furthermore, the mechanism involved in spontaneous LT is different from that of iodine-induced LT in NOD mice.


INTRODUCTION

Hashimoto’s thyroiditis (HT) is a well-known autoimmune thyroid disease characterized by mononuclear cell infiltration in the thyroid and production of autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg) (1). Intrathyroidal infiltrating cells are mainly CD4+ and CD8+ T cells (2, 3), suggesting the involvement of these cells in the production of anti-thyroid antibodies and destruction of follicles. However, their importance in the pathophysiology of HT remains largely undefined.

Lymphocytic thyroiditis (LT) in non-obese diabetic (NOD) mice serves as a model of HT in humans (4). Although NOD mice spontaneously develop LT, thyroid lesions are usually mild and occur in a relatively small percentage of the mice (5, 6). In contrast, iodine administration results in increased frequency and severity of LT in NOD mice (7, 8). Thus, iodine-treated NOD mice rather than those without any treatments are usually used in many studies. Previous studies have demonstrated the infiltration of CD4+ and CD8+ T cells in the thyroid of iodine-treated NOD mice (7, 9). Both CD4+ and CD8+ T cells are required for iodine-accelerated LT, since depletion of these cells prevented LT in NOD and NOD-H2h4 mice (7, 9, 10). CD4+ T cells are pivotal for both the initiation and maintenance of the disease, while CD8+ T cells are temporally required at an early stage in the disease process (9, 10). Thus, these studies suggest that CD8+ T cells play a role different from that of CD4+ T cells in the development of iodine-induced LT. In contrast, the function of CD4+ and CD8+ T cells remains unclear in the spontaneous form of LT in NOD mice.

Recently, we have shown that iodine administration fails to induce LT in interferon regulatory factor-1 (IRF-1) knockout NOD mice, demonstrating that

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IRF-1 plays a critical role in the development of iodine-induced LT in NOD mice (8). IRF-1 is a transcription factor involved in the interferon-mediated immune reaction, differentiation of CD8+ T cells, and development of T helper 1 (Th1) immune response (11). In fact, IRF-1 deficient mice have markedly decreased number of CD8+ T cells in the thymus and peripheral lymphoid organs (12, 13). These findings indicate the usefulness of IRF-1 deficient NOD mice in the study to elucidate the pathogenesis of murine LT. Accordingly, in the present study, we used IRF-1 +/+ , +/- and −/− NOD mice to determine the role of IRF-1 in the spontaneous development of LT. Our results demonstrate that LT can develop spontaneously in IRF-1 deficient NOD mice without CD8+ T cell infiltration in the thyroid, suggesting that the mechanism involved in spontaneous LT is different from that of iodine-induced LT in NOD mice.

MATERIALS AND METHODS

Mice

NOD mice with a disrupted IRF-1 gene were generated and characterized as previously described (14). All mice were genotyped and maintained in our animal facility under specific pathogen free conditions. Sera, thyroids and spleens were obtained at 24 weeks of age. This study was approved by the institutional review board of Tohoku University School of Medicine.

Histopathology

Thyroids were fixed in 4% phosphate-buffered paraformaldehyde solution and embedded in paraffin. Four μm-thick sections were taken at 6 to 10 levels in a non-contiguous way and stained with hematoxylin and eosin. Thyroids were scored quantitatively for LT, defined as the percentage of thyroid infiltrated, using a scale as described previously (15): 0=normal thyroid; 1=less than 10% lymphocytic infiltration of the thyroid; 2=10-30% lymphocytic infiltration; 3=30-50% lymphocytic infiltration; 4=greater than 50% lymphocytic infiltration. The histological specimens were interpreted by 2 investigators in a blind fashion. The total score for each mouse was divided by the number of observations for that mouse.

Immunohistochemistry

Cryostat sections (5 μm thick) of thyroids, fixed in 4% phosphate-buffered paraformaldehyde solution, were incubated with 10% normal goat serum and then with either biotin-conjugated anti-mouse CD4 (Caltag, South San Francisco, CA), biotin-conjugated anti-mouse CD8 (Caltag), or biotin-conjugated anti-rat RT1B (OX6; PharMingen, San Diego, CA) which interacts with MHC class II Aβ. After endogenous peroxidase was inactivated with methanol containing hydrogen peroxide, the sections were incubated with streptavidin-conjugated horseradish peroxidase (Caltag). The immunoreaction was visualized with 3,3′-diaminobenzidine (Dojin, Kumamoto, Japan), and then the sections were counterstained with hematoxylin. Negative controls were done as above with omission of the primary antibody, and the absence of positive staining was confirmed in the controls (data not shown).

Anti-mouse Tg antibody and serum T₄

Mouse Tg (mTg) was prepared as previously described (16). Serum anti-mTg antibody (Ab) levels were determined as previously described (8). Results are expressed as absorbancy minus the reagent blank. Serum T₄ was measured by enzyme immunoassay with reagents supplied by Dainabot Co. (Tokyo, Japan).

Flow cytometry

Cell surface phenotypes were analyzed by FACSCalibur and the CellQuest software (Becton Dickinson, Mountain View, CA) as previously described (8). Spleen cells were stained with either FITC-conjugated anti-CD3 or FITC-conjugated anti-B220 (Caltag). Cells stained with the former were further incubated with either APC-conjugated anti-CD4 or PerCP-labeled anti-CD8 (PharMingen). Ten thousand cells were counted.

In vitro proliferation and cytokine assay

As previously described (8), spleen cells were cultured in flat-bottomed, 96-well tissue culture plates at a concentration of 5x10⁵ cells/well in the presence or absence of mTg (40 μg/ml) or concanavalin A (ConA) 1 μg/ml, Sigma Chemical Co., St. Louis, MO). Following 66 h of incubation, 1 μM bromodeoxyuridine (BrdU; Amersham Pharmacia Biotech, Tokyo, Japan) was added, and cells were further incubated for 6 h. BrdU incorporated into spleen cells was determined using an ELISA kit (Amersham Pharmacia Biotech). IFN-γ and interleukin-10 (IL-10) levels in 72-h supernatants were measured using ELISA kits (Biosource International, Camarillo, CA).

Statistical analysis

The incidence of LT was tested by Fisher’s exact probability test. The severity of LT was compared by one-way analysis of variance (ANOVA) followed by the Kruskal-Wallis test. Serum anti-mTg Ab levels are shown as the median (range) and were compared by the same test as the severity of LT. The other data are shown as the mean±SD and were compared by unpaired Student’s t-test or one-way ANOVA followed by Bonferroni test. A level of p<0.05 was considered statistically significant.

RESULTS

As shown in Table 1, IRF-1 +/+ , +/- and −/− NOD mice developed LT at 24 weeks of age. Noticeably, mild thyroid lesions (severity score 1) were observed in most mice (Fig. 1 and 2). There were no differences in the incidence and severity of LT among three IRF-1 genotypes (Table 1; Fig. 2). In addition, no sex-associated difference was found in the incidence and severity of LT (data not shown). Serum anti-mTg Ab and T₄ levels were not different among the three IRF-1 genotypes (Table 1). Thyroids of IRF-1 +/+ and −/− mice were examined immunohistochemically to determine the composition of thyroid infiltrates and MHC class II antigen expression. In IRF-1 +/+ mice, both CD4+ and CD8+ T cells were demonstrated in the area of lymphocytic infiltration (Fig. 3). CD4+ T cells were predominant cells in the inflamed thyroid. In contrast, CD8+ T cells were not detected in thyroid infiltrates of IRF-1 −/− mice, while accumulations of...