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# Relationship between induction of an experimental autoimmune thyroiditis and deglycosylation of thyroglobulin

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## Abstract

Experimental autoimmune thyroiditis (EAT), the murine model of human hashimoto's thyroiditis, is an organ-specific autoimmune disease which can be induced in genetically susceptible strains of mice by immunization with autologous or xenogeneic thyroglobulin (Tg), including particular peptides in human Tg . It was known that denaturation of exogenous antigen (Ag) induces CTL. Immunization of naive CBA/J mice with heat-denatured porcine Tg (hdpTg) induced EAT with similar kinetics to conventional EAT induced by Tg and adjuvants, and with apparent differences in CTL and IFN-$\gamma$ as acting mediators, as follows: 1) hdpTg-induced EAT was prevented by in vivo treatment with mAB directed against CD8+ T cells or IFN-$\gamma$. 2) Ab titers to pTg and IgG1:IgG2a ratio were lower in sera from hdpTg-immunized mice than

in sera from conventionally immunized mice, demonstrating this EAT is mediated by CD8+ CTL, producing IFN-$\gamma$, a cell subset named Tc1.

Contribution of cytotoxic T lymphocytes (CTL) to experimental autoimmune thyroiditis (EAT) was well defined. However, there is no any report on a difference between glycosylated and non-glycosylated form thyroglobulin (Tg), although Tg is a high molecular weight glycosylated iodoprotein (660 kDa), which is the site of synthesis and storage of the thyroid hormone, thyroxine. The native Tg showed high sensitivity to endo-$\beta$-N-acetylglucosaminidase F (Endo F) and its molecular weights, corresponding to about 330 kDa as a monomer and 660 kDa as a dimer, was reduced to slightly smaller molecular weight forms by treatment of Endo F and trifluoromethanesulfonic acid (TMSF), suggesting N-glycosylation type. Then, porcine thyroglobulin (pTg) was classifed into the following 2 categories: 1) deglycosylated porcine Tg (dgpTg) by N-glycanase F and TMSF treatment and 2) native pTg. They were injected i.v. into CBA/J mice, without the aid of adjuvants. Both lymphocytic infiltrations of the thyroid glands and levels of Tg-specific CTL were similar to those found in conventional EAT induced by Tg and adjuvants. In contrast, proliferative responses in native pTg and dgpTg-injected mice could not be detected, and titers of antibodies to pTg and dgpTg were 20 times and 30 times lower than that of pTg and adjuvants, respectively. These EAT-inducer CTL belong to the CD8+ cell subset and exerted their thyroiditogenic potential through release of IFN-$\gamma$. We conclude that dgpTg-induced EAT are mediated by type 1 cytotoxic T cells (Tc1). Also, results that EAT induction of the glycosylated pTg (gpTg) was much lower than that of dgpTg, suggested that the abberant and incomplete glycosylation of the thyroglobulin is responsible for the induction of autoimmune thyroiditis.