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Nonimmune Thyroid Destruction Results from Transgenic Overexpression of an Allogeneic Major Histocompatibility Complex Class I Protein

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The overexpression of major histocompatibility complex (MHC) class I molecules in endocrine epithelial cells is an early feature of autoimmune thyroid disease and insulin-dependent diabetes mellitus, which may reflect a cellular response, e.g., to viruses or toxins. Evidence from a transgenic model in pancreatic β cells suggests that MHC class I overexpression could play an independent role in endocrine cell destruction. We demonstrate in this study that the transgenic overexpression of an allogeneic MHC class I protein (H-2K^b) linked to the rat thyroglobulin promoter, in *H-2K^k* mice homozygous for the transgene, leads to thyrocyte atrophy, hypothyroidism, growth retardation, and death. Thyrocyte atrophy occurred in the absence of lymphocytic infiltration. Tolerance to allogeneic class I was revealed by the reduced ability of primed lymphocytes from transgenic mice to lyse *H-2K^b* target cells in vitro. This nonimmune form of thyrocyte destruction and hypothyroidism recapitulates the β-cell destruction and diabetes that results from transgenic overexpression of MHC class I molecules in pancreatic β cells. Thus, we conclude that overexpression of MHC class I molecules may be a general mechanism that directly impairs endocrine epithelial cell viability.

The overexpression of major histocompatibility complex (MHC) class I molecules is an early feature of the pathology in autoimmune diseases, e.g., in pancreatic islet β cells in insulin-dependent diabetes (IDD) and thyrocytes in Graves' disease (17, 19). This feature can be present in the absence of an inflammatory cell infiltrate, suggesting that it may be independent of and precede the effects of cytokines released from infiltrating mononuclear cells (4, 17). The finding of immunoreactive alpha interferon in the early lesion of IDD could provide an explanation for the overexpression of MHC class I molecules and might itself reflect the presence of underlying virus infection (18). The direct infection of cultured human pancreatic islet cells by reovirus (9) or of rat thyrocytes by simian virus 40 (3) causes upregulation of MHC class I expression. In endocrine autoimmunity, mononuclear cells infiltrate the target tissue and release cytokines such as gamma interferon and tumor necrosis factor, which may further upregulate MHC class I and induce MHC class II molecules in islet cells (6, 10–12, 33) and thyrocytes (22, 24, 28, 32). The overexpression of MHC class I molecules should thus enhance the immunogenic properties of endocrine cells (8, 19).

In mice syngeneic or allogeneic to *H-2K^b*, transgenic overexpression of the MHC class I molecule H-2K^b in β cells leads to a defect in insulin secretion, β-cell destruction, and diabetes in the absence of lymphocytic infiltration (1). This finding is consistent with a direct, nonimmune role for MHC class I molecules in the destructive process (20). β cells appear to be especially susceptible to MHC molecules expressed transgenically (1, 23, 34) because the expression of other transgenes in the β cell that encode several cell surface or nuclear proteins does not, in general, lead to diabetes (20). The mechanism of this deleterious effect of MHC overexpression on differentiated cell function and viability remains unclear (30). The normal function of MHC class I molecules is to associate with endogenous cell peptides and present them to T cells bearing the CD8 accessory molecule (5). Therefore, as previously postulated (20), overexpressed MHC class I molecules might associate promiscuously with key cellular proteins and impair cell function. Even though the mechanism and specificity of the effect is still a matter of conjecture, this does not diminish the potential significance of MHC class I overexpression as a mediator of pathological change. To demonstrate whether the deleterious effect of MHC class I overexpression might apply more generally to differentiated endocrine epithelial cells, we have created a transgenic model of MHC class I overexpression in the thyroid, the most common site of human autoimmune disease. To express MHC class I transgenically in the thyroid, the mouse *H-2K^b* gene was linked to the thyroid-specific rat thyroglobulin promoter (TP) and injected into fertilized eggs from (CBA × B10.BR)F_2 mice (*H-2K^k* background haplotype).

MATERIALS AND METHODS

Construction of a TP-*H-2K^b* construct. The TP was kindly provided by Roger Cone (Vollum Institute, Portland, Ore.). The *H-2K^b* genomic clone in a pUC18 vector (pK^{b}6) was previously described (1, 38). The rat genomic TP from base positions −684 to +175 was inserted at the *Sal*I site of the Bluescribe$^-$ vector. Oligonucleotides (28-mer) were designed (Joint Protein Structure Laboratory, Ludwig Institute/Walter and Eliza Hall Institute) to include a new *Hin*dIII site without the *Sal*I site at the 5′ end of the promoter (−684) and a new *Sal*I site at the 3′ position (+38). Using these oligonucleotides as primers, polymerase chain reaction (PCR) yielded an appropriate-size fragment. This PCR product was purified from a 1% low-melting-point agarose gel. The TP PCR fragment and the mouse genomic MHC class I (*H-2K^b*) gene (pK^{b}6) (38) were then separately digested with

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*Hin*dIII and *Sal*I, combined, and reacted with T4 DNA ligase at 15°C for 18 h. Competent *Escherichia coli* JPA 101 cells were transformed with the ligase reaction and plated on LB plates, and individual colonies were picked and transferred to nitrocellulose filters placed on fresh LB plates. Colony lysis and hybridization with the ^{32}P-labeled TP PCR fragment revealed strong signals in approximately 45% of colonies on autoradiography. Colonies from three of the strongest positive clones contained the TP with the *H-2K^b* gene in the correct orientation, as confirmed by multiple restriction digests and direct sequencing. The TP-*H-2K^b* fusion gene was then digested with *Hin*dIII and *Xho*I, electroeluted from a 0.6% agarose gel, chloroform extracted, ethanol precipitated three times, and then dissolved in injection buffer (10 mM Tris [pH 7.4], 0.1 mM EDTA).

Microinjection and screening of fusion construct. The fusion construct (6.2 kb) was injected at 2 μg/ml into (CBA × B10.BR)F_2 mouse pronuclei (*H-2K^k* haplotype). Embryos were then transferred to the oviducts of CBA × C57BL/6 pseudopregnant mice. Mice were screened by tail DNA analysis. At the time of weaning (3 to 4 weeks of age), 1 cm of the tail tip was cut from all mice, sheared with fine scissors, digested by overnight incubation in proteinase K (500 μg/ml), and subjected to phenol-chloroform extraction. An aliquot was then digested overnight with *Bam*HI and run on a 0.8% agarose gel. The gel was washed and transferred to a nylon filter (Zetaprobe; Bio-Rad), which was then probed with ^{32}P-labeled TP, washed, and exposed for autoradiography. Copy number of the transgene was estimated by densitometry of autoradiographs (using a computing densitometer and analysis by MD Imagequant software, version 3.2, both from Molecular Dynamics, Sunnyvale, Calif.), comparing the intensities of the bands representing transgenic (rat) and endogenous (mouse) TPs. The efficiency of hybridization of the rat TP probe to the mouse and rat TPs was established after Southern analysis of equal amounts (10 μg) of *Bam*HI-digested mouse and rat genomic DNA and densitometry of autoradiographs. The rat TP probe was, by this method, estimated to hybridize mouse DNA with 70% efficiency compared with rat DNA. This value was used as a correction factor in determining the transgenic copy number. Mice were bled initially every 4 weeks for analysis of serum-free thyroxine (T_4) levels measured by a routine two-step radioimmunoassay (Gamma Coat ^{125}I Free T_4 RIA; Baxter Healthcare Corp., Cambridge, Mass.).

Histology and immunofluorescence. Animals were sacrificed at various times, and the tissues (thyroid, thymus, spleen, pancreas, kidney, and liver) were fixed in Bouin's solution for routine histologic analysis or snap frozen in OCT compound (Miles Inc. Diagnostic Division, Elkhart, Ind.) for immunohistologic studies. For immunofluorescence, 5-μm sections were cut with a cryostat. Sections were then fixed in acetone on gelatinized slides and stored at −20°C until use. Initially, sections were thawed at room temperature and washed in phosphate-buffered saline (PBS). Blocking solution (1% fetal calf serum [FCS] in PBS) was applied over the sections at room temperature for 10 to 20 min. TIB 139 (1), a mouse monoclonal antibody specific for H-2K^b (50 μl in blocking solution), was applied for 1 to 2 h. PBS washes were performed three times, and second antibody (1:150 fluorescein isothiocyanate [FITC]-conjugated anti-mouse immunoglobulin; Silenus Laboratories, Hawthorn, Victoria, Australia) was applied for 30 min. Further washes were performed, and slides were covered with a coverslip and examined under green fluorescence (Axiophot microscope; Zeiss).

Ultrastructural changes in the tissues were evaluated by electron microscopy. Organs were immersion fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate (pH 7.4) overnight at 4°C and then postfixed in 2% OsO_4 for 60 min at room temperature. Tissues were then stained with 4% aqueous uranyl acetate and dehydrated through a graded acetone series, embedded in resin, and polymerized for 8 h at 65°C. Sections were cut, stained with 10% uranyl acetate–Reynold's lead citrate, dried, and viewed on a Philips CM12 transmission electron microscope at 60 kV.

Quantitation of transgene expression. Two independent methods were used to estimate the amount of transgenic protein expression. First, immunofluorescence sections were scanned with a computing densitometer, and images were analyzed by MD Imagequant software, version 3.2 (Molecular Dynamics). Fifteen follicles from each of two different sets of thyroid sections (from nontransgenic, heterozygous transgenic, and homozygous transgenic mice) were scanned and analyzed. Statistical analysis was performed by paired Student's *t* test. Second, fluorescence-activated cell sorting (FACS) analysis was performed on single-cell suspensions of thyrocytes prepared from the thyroids of heterozygous transgenic mice and nontransgenic littermates and of control CBA (*H-2K^k*) and C57BL/6 (*H-2K^b*) mice. Thyroids were overdigested in collagenase type IV (5 mg/ml; Worthington, Freehold, N.J.) in RPMI containing 10% FCS and 10^{-5} M 2-mercaptoethanol. Spleen single-cell suspensions were prepared as described below for the cytotoxic T-lymphocyte assay; thymus single-cell suspensions were prepared in a similar fashion. Cell suspensions were washed once in FACS buffer (2% FCS–0.02% sodium azide in PBS) and incubated for 30 min at 4°C with the primary antibody or the FACS buffer as a negative control. The primary antibodies were TIB 139, hybridoma medium containing anti-H-2K^k monoclonal antibody, A112F (1), and a control monoclonal antibody (9GB5) against reovirus at 1:100 (7). After being washed in FACS buffer, the cells were incubated with a FITC-conjugated anti-mouse immunoglobulin (1:40) for 20 min at 4°C, washed, and resuspended in 500 μl of FACS fixative (2.5% formaldehyde–2% glucose–0.01 mM sodium azide in PBS). Cells (10^4 to 10^5 per sample) were analyzed by flow cytometry on a FACS II scanner (Becton Dickinson Electronics Laboratory, Mountainview, Calif.), using green fluorescence.

Cytotoxic T-lymphocyte assay. Single-cell suspensions containing a mixture of cells from lymph nodes (axillary, mesenteric, and inguinal) and spleens of heterozygous transgenic mice and nontransgenic littermates and of control CBA (*H-2K^k*) and C57BL/6 (*H-2K^b*) mice were prepared. Organs were pushed through a sieve and washed once with PBS, and erythrocytes were lysed by a hypotonic solution (0.01 M $NaHCO_3$, 0.1 mM Na_4 EDTA, 0.15 M NH_4Cl [pH 7.3]). Cells were then washed twice in medium (RPMI with 10% FCS and 2-mercaptoethanol) and resuspended to a final concentration of 2×10^6/ml. These effector cells were primed for 5 days in vitro against irradiated (2,000 rads) B10.A(5R) (*H-2K^{b}D^d*) splenic lymphocytes (stimulator cells) in a 1:1 ratio. Target cells, EL4 (*H-2^b*) and control P815 (*H-2^d*) murine lymphoma cells, were labeled with 200 μCi of ^{51}Cr in 200 μl of RPMI. The labeled target cells were then centrifuged, washed, resuspended to 2×10^5 cells per ml, and incubated with primed effector cells for 4 to 6 h at effector/target ratios of 1:1, 10:1, 50:1, and 100:1. Lysis of target cells was measured by counting γ radioactivity in culture supernatants. Counts from target cells incubated in medium alone or with 1% Triton X-100 determined basal

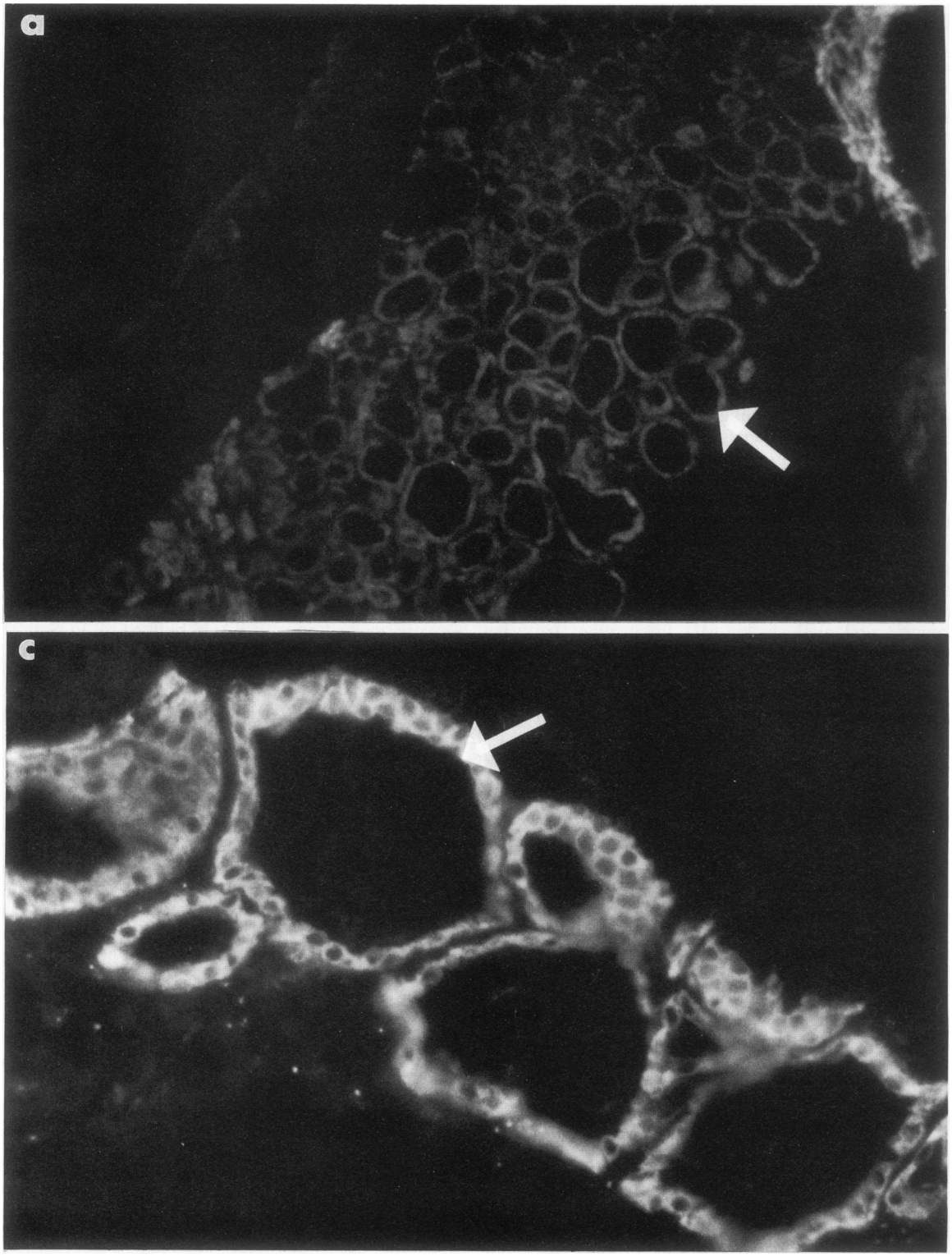

FIG. 1. (a and b) Immunofluorescence staining for H-2K^b protein in thyroid from a heterozygous TP-*H-2K^b* transgenic mouse (a) and a nontransgenic littermate (b) (magnification, ×200; lineage 159-7, 6 months of age). The arrow indicates a thyroid follicle. (c and d) Immunofluorescence staining for H-2K^b protein in thyroid from a homozygous TP-*H-2K^b* transgenic mouse (c) and a heterozygous transgenic littermate (d) (magnification, ×400; lineage 159-7, 2 weeks of age; results for mice of identical lineage and age are shown in Fig. 3 to 5). The arrow indicates a thyroid follicle.

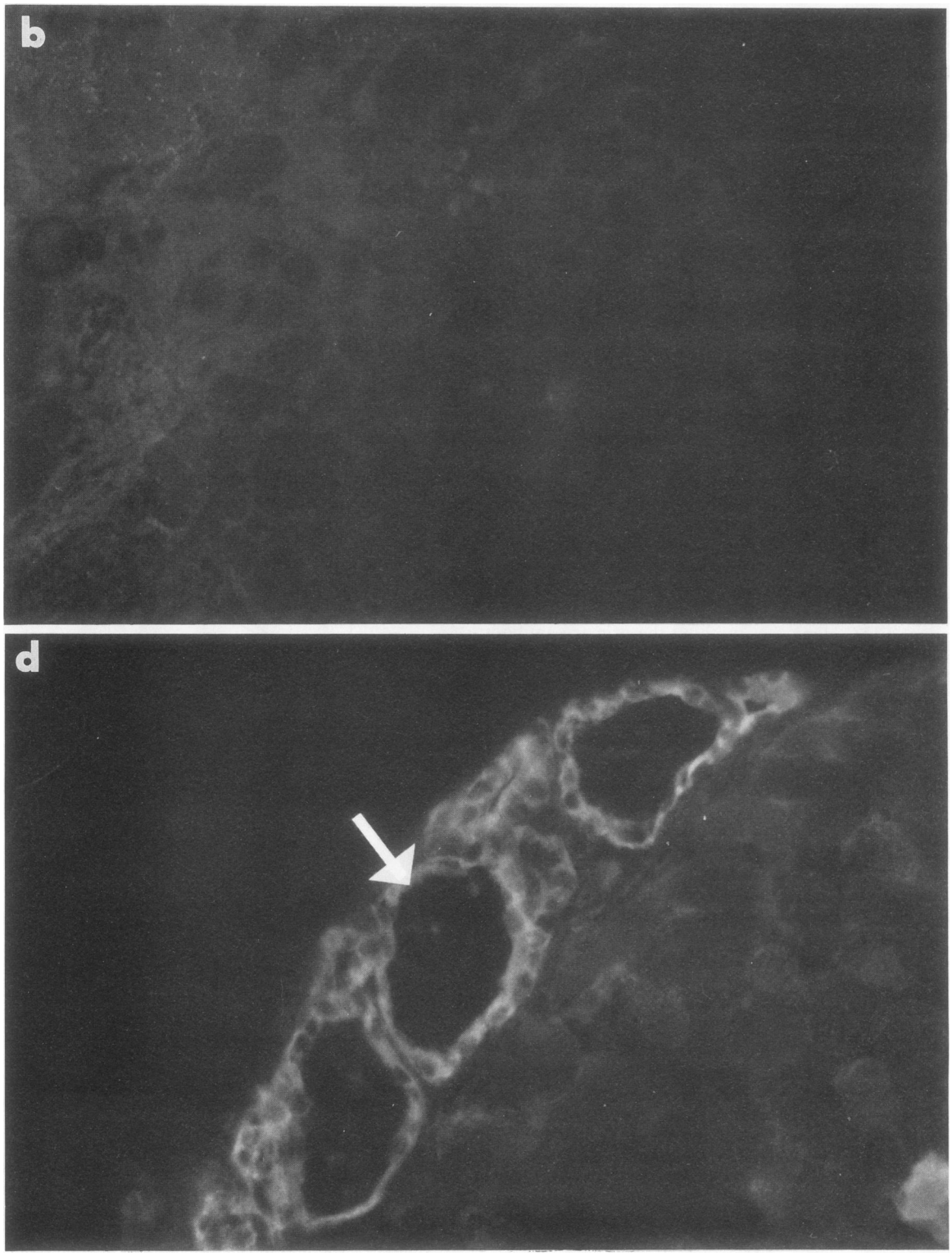

FIG. 1—*Continued*.

(<10% total) and maximal lysis, respectively. Specific lysis was calculated as [(cpm with effector cells − basal cpm)/(maximal cpm − basal cpm)] × 100.

RESULTS

Heterozygous TP-*H-2K*^{b} transgenic mice. In the offspring of the mice carrying the injected embryos, five litters produced 32 mice, of which 8 were positive for the transgene on Southern analysis. Three of these eight founder transgenic mice were crossed with CBA mice to establish subsequent transgenic generations. Copy numbers of the first-generation mice were as follows: line 159-7 (selected for inbreeding), 12; line 160-9, 2; and line 160-12 (selected for inbreeding), 6. The eight founder mice developed normally in terms of physical activity, feeding, and weight gain and remained phenotypically normal at 12 months of age. Immunofluorescence at 6 months of age confirmed the overexpression of H-2K^b in the thyrocytes of transgenic mice (Fig. 1a) but not their nontransgenic littermates (Fig. 1b). Other organs of the transgenic mice (thymus, spleen, pancreas, kidney, and liver) did not demonstrate H-2K^b expression (data not shown). Histology in the founder generation of transgenic mice revealed the absence of a mononuclear cell infiltrate and a preserved thyroid follicular structure. No significant phenotypic, thyroid histologic or serum-free T_4 changes were seen in three subsequent generations of heterozygous mice.

FACS analysis demonstrated surface expression of H-2K^b molecules on thyrocytes of heterozygous transgenic but not nontransgenic mice, the mean fluorescence intensities being 30.5 and 19.6 arbitrary units, respectively. The latter value was comparable to that seen in thyrocytes incubated with anti-mouse FITC alone, i.e., without the primary antibody. Thymic and splenic cells from transgenic and nontransgenic mice showed H-2K^k but not H-2K^b expression compared with cells incubated without primary antibody. No cells expressed control (reovirus) antigen.

Splenic lymphocytes from heterozygous transgenic mice primed in vitro against H-2K^b were significantly less effective at lysing ^{51}Cr-labeled EL4 (*H-2*^{b}) cells compared with primed, nontransgenic lymphocytes (Fig. 2a). Transgenic and nontransgenic lymphocytes lysed control P815 (*H-2*^{d}) target cells equally (Fig. 2b). Primed lymphocytes from control CBA (*H-2K*^{k}) mice showed, as expected, significant lysis of EL4 and P815 cells, whereas lymphocytes from C57BL/6 (*H-2K*^{b}) mice lysed P815 but not EL4 target cells (data not shown).

Homozygous transgenic mice. Mice homozygous for the transgene were created by inbreeding the two lines of third-generation heterozygous transgenic mice with the highest transgene copy number (lines 159-7 and 160-12). Litter number, birth weight, and feeding of offspring of these matings initially appeared normal. However, after 1 to 2 weeks, it was evident that one-third to one-half of the mice were small, being only about 50% of the body weight of normal-size littermates; by 2 to 3 weeks of age, these small mice had become sluggish, and by 3 weeks of age, all had died. These mice were homozygous for the transgene and had lower serum-free T_4 concentrations than did their larger, heterozygous littermates (11.1 ± 2.9 versus 24.3 ± 8.3 pmol/liter; $P < 0.001$, $n = 6$). Immunofluorescence staining confirmed the presence of H-2K^b in the thyrocytes of these mice (Fig. 1c), the expression of which was greater than in their heterozygous littermates (Fig. 1d). Quantitation of transgene expression by image analysis of immunofluorescence staining revealed average intensities of 315 ± 28, 541 ± 24, and 1,375 ± 253 arbitrary units (mean ± standard error of the mean, $n = 15$ follicles, line 159-7) for representative nontransgenic, heterozygous transgenic, and homozygous transgenic thyroid sections, respectively ($P < 0.01$ for homozygous versus heterozygous and for heterozygous versus nontransgenic values).

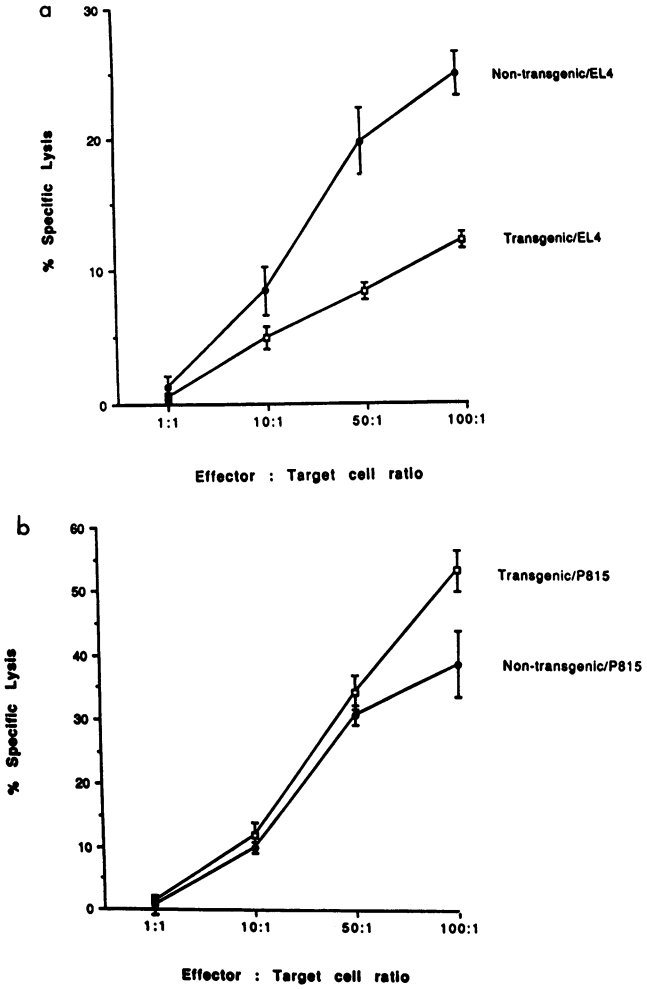

FIG. 2. Cytolytic activity of *H-2K*^{b}-primed splenic lymphocytes (effector cells) from heterozygous transgenic and nontransgenic littermates on ^{51}Cr-labeled EL4 (*H-2*^{b}) (a) and P815 (*H-2*^{d}) (b) target cells. Results shown are means ± standard deviations of triplicates from a representative experiment.

Histologic examination of the thyroid from homozygous mice (Fig. 3a) revealed reduction in follicular size and atrophy of thyrocytes compared with heterozygous littermates (Fig. 3b) and an absence of lymphocytic infiltration. Electron microscopy revealed reduction in thyrocyte height, increased density of thyrocyte cytoplasm and nuclei, and disorganized follicles in homozygous (Fig. 4a) but not heterozygous (Fig. 4b) mice. Furthermore, thyrocytes from homozygous mice had distended and irregular rough endoplasmic reticulum in the presence of normal Golgi structures (Fig. 5a) compared with heterozygous mice (Fig. 5b). These phenotypic, biochemical, and histologic changes have been seen in two independent lineages of homozygous transgenic mice.

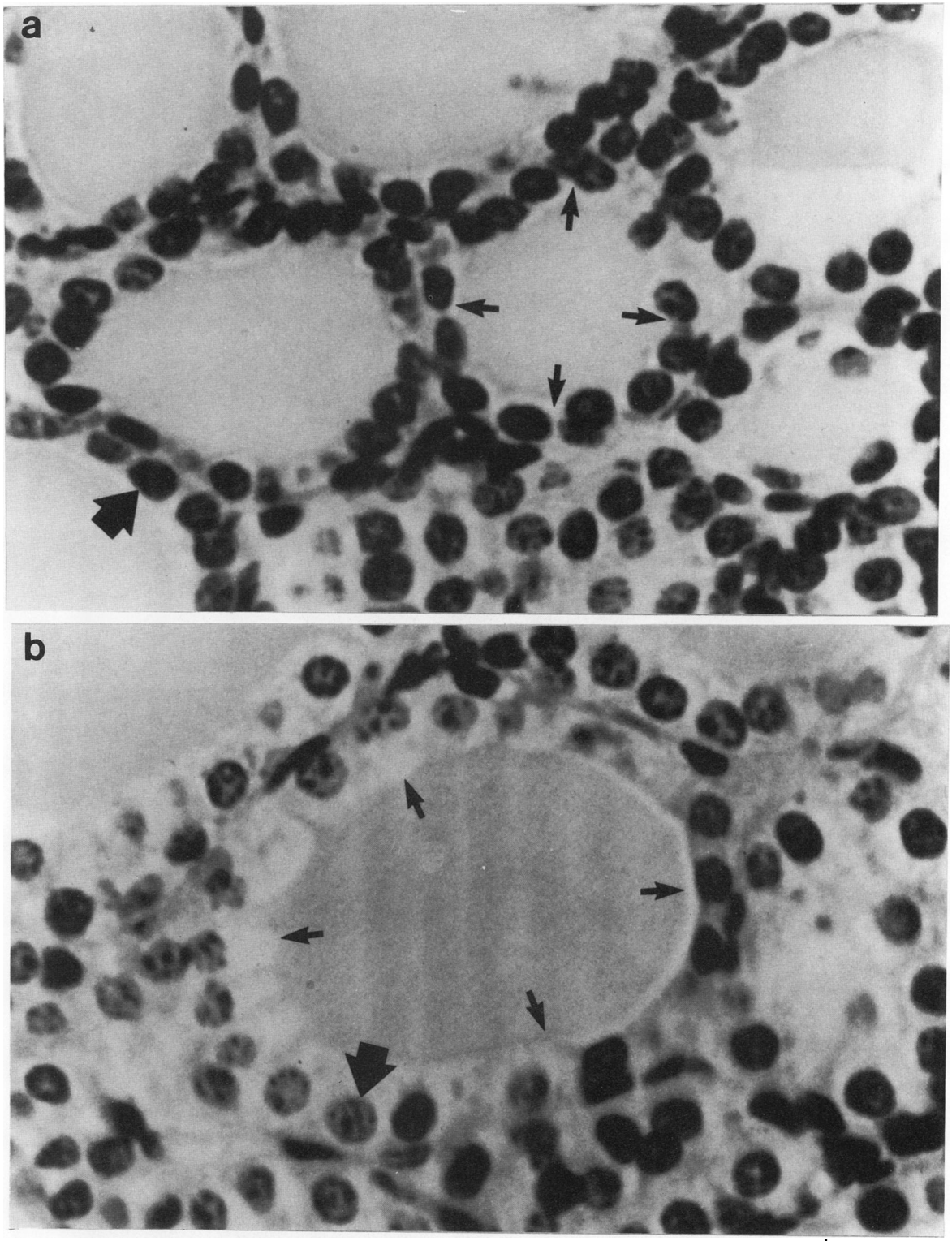

FIG. 3. Hematoxylin and eosin staining of thyroid from homozygous (a) and heterozygous (b) TP-*H-2K*^{b} transgenic littermates (magnification, ×1,000). Narrow arrows, follicles; broad arrow, follicular cell.

DISCUSSION

The present findings for thyroid transgenic mice, taken together with those previously reported for β-cell transgenic mice (1), in which *H-2K*^{b} was linked to the rat insulin promoter (RIP-*K*^{b}), lead to the general conclusion that overexpression of an MHC class I molecule is detrimental to endocrine epithelial cell viability. The possible role of MHC class I overexpression in nonendocrine disease is under-

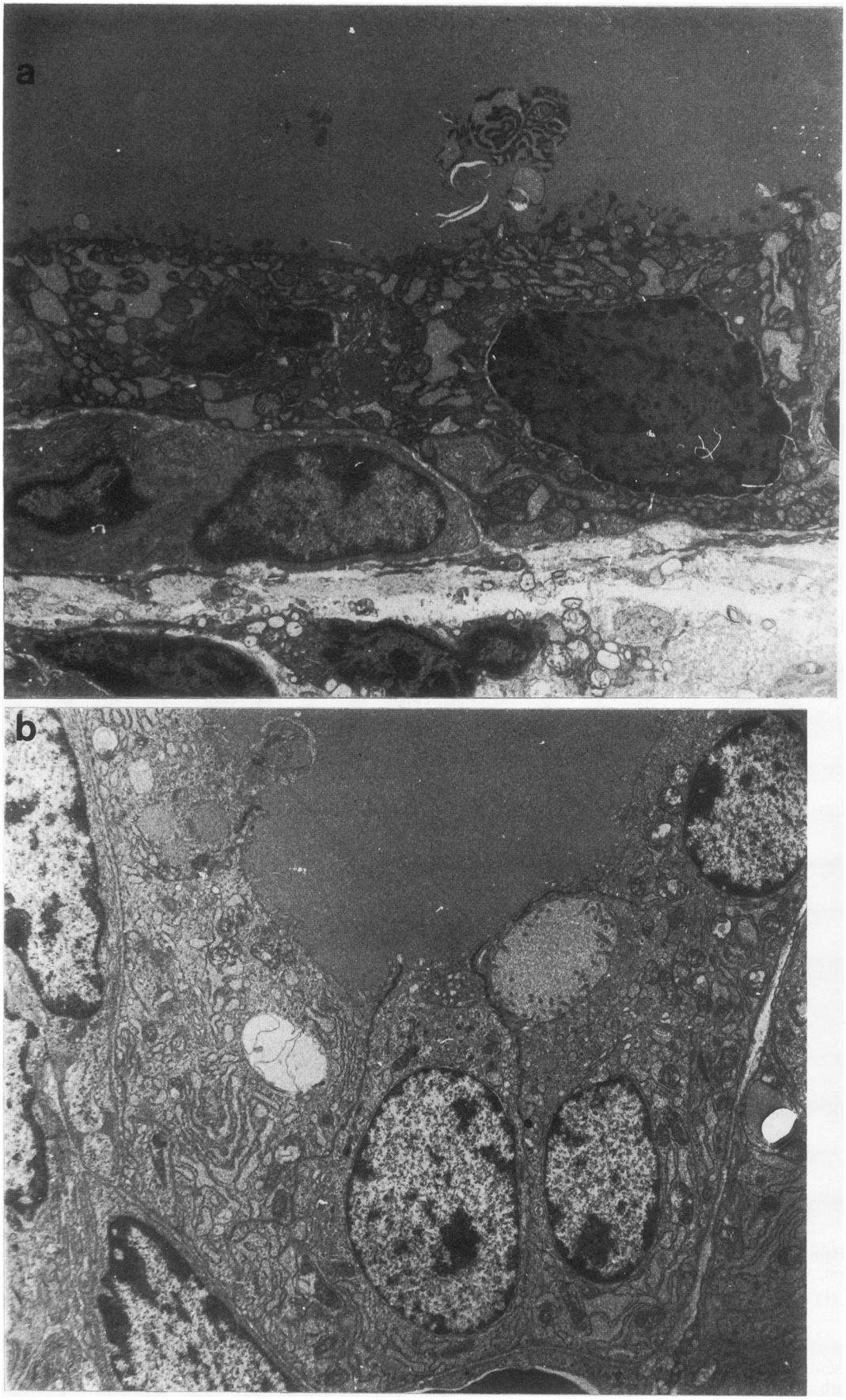

FIG. 4. Electron microscopy of thyroid from homozygous (a) and heterozygous (b) TP-*H-2K*^{b} transgenic mice (magnification, ×4,600).

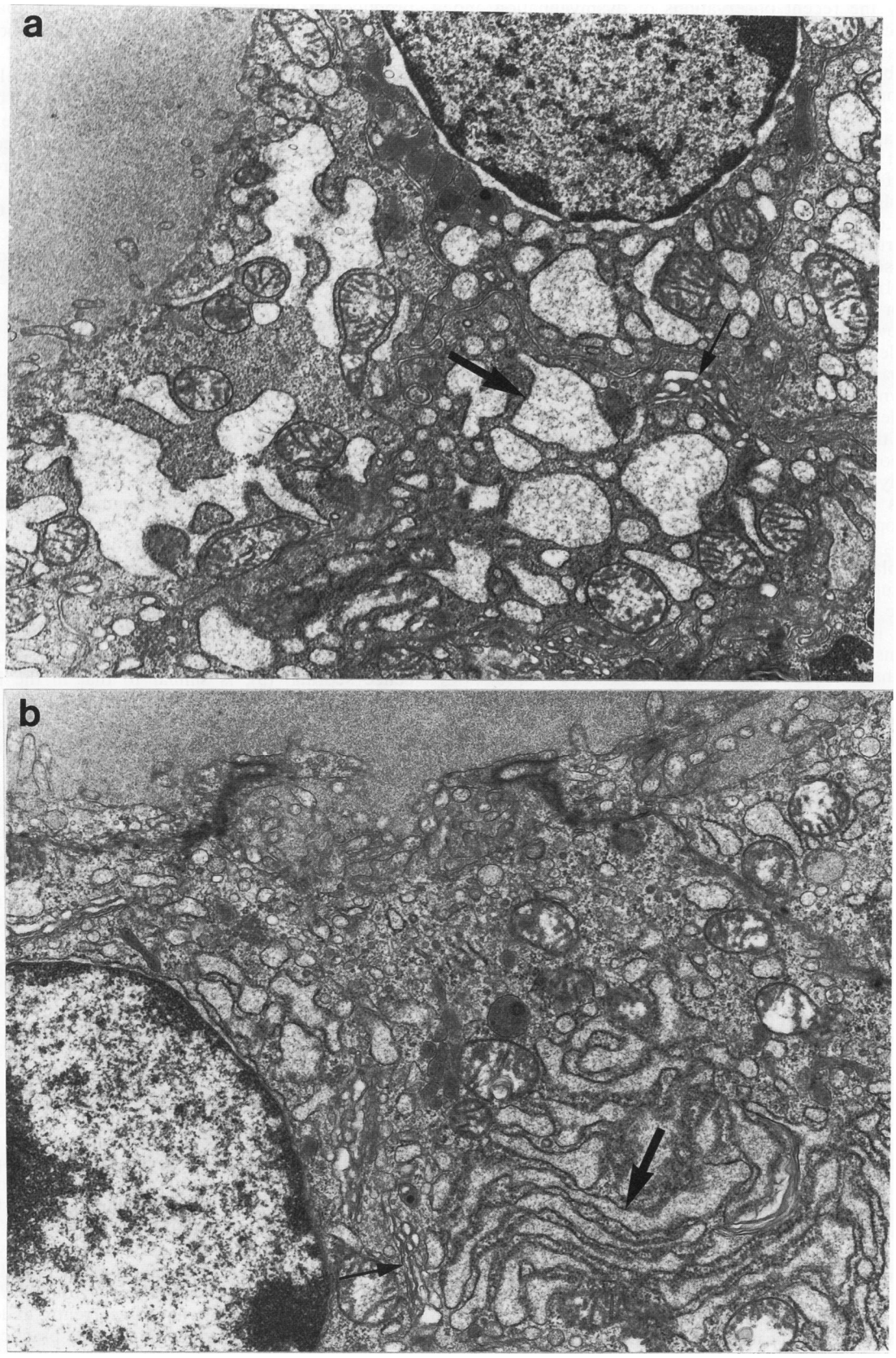

FIG. 5. Electron microscopy of thyroid tissue from homozygous (a) and heterozygous (b) TP-*H-2K*^{b} transgenic mice (magnification, ×8,000). Broad arrows, endoplasmic reticulum; narrow arrows, Golgi complex.

scored by the recent observations of dysmyelination and neurological impairment in transgenic mice overexpressing MHC class I molecules in oligodendrocytes (36). The mechanism(s) by which the transgenic overexpression of MHC class I impairs cell viability deserves further investigation because of the potential importance of MHC class I overexpression as a mediator of pathology in early autoimmune disease. It has been speculated that integration of transgenes may interfere with transcription of normal genes (31). However, the likelihood of transgene integration into a thyroid-specific, i.e., thyroglobulin, site in the genome is extremely small. Furthermore, the fact that similar thyroid abnormalities were seen in two separate mouse lineages argues against this idea. Interference with synthesis or transport of proteins critical for cell function may explain the deleterious effects of MHC overexpression in transgenic models. The presence of distended, irregular endoplasmic reticulum with an intact Golgi apparatus in the thyroid favors a defect at the level of protein synthesis or early export. Overexpressed MHC class I molecules might interfere directly with the synthesis or folding of thyroglobulin, making it incapable of or unsuitable for transport from the endoplasmic reticulum. Furthermore, it is likely that the transgenic overexpression of H-2K^b molecules results in their improper assembly and export from endoplasmic reticulum because of limiting amounts of (native) β_2 microglobulin. Thus, K^b heavy chains trapped in the endoplasmic reticulum could impair cell function. This idea is supported by the observation that mice doubly transgenic for RIP-*K^b* and RIP-β_2 microglobulin have less abnormal islet morphology and insulin secretion than do single transgenic RIP-*K^b* mice (2).

The transgenic overexpression of MHC class I molecules might outstrip the capacity of transporter proteins that supply peptides to MHC molecules. Mutant B-cell lines defective for the expression of Tap (transporter associated with antigen processing) proteins are unable to transport peptides from the cytoplasm into the endoplasmic reticulum for binding to MHC class I molecules (26). MHC class I molecules purified from these cells have been found to contain bound signal peptide sequences (37). This observation implies that in the absence of a coordinately regulated peptide supply, newly synthesized MHC class I molecules bind adventitiously to peptides in the local environment. Another mechanism for cellular dysfunction could be competition between the transgenic and endogenous thyroglobulin promoters for transcriptional factors. This seems less likely because there was no relationship between copy number and disease in RIP-*K^b* mice, and it is difficult to see how this would explain the appearance of the endoplasmic reticulum. The development of thyroid atrophy in the homozygous and not heterozygous mice suggests that a critical concentration of transgenic H-2K^b protein is required to interfere with cell function at the level of the endoplasmic reticulum. Finally, it is possible that competition between overexpressed class I molecules and key cellular proteins for binding to a common chaperonin leads to disruption of critical cellular functions. Class I molecules are associated in the endoplasmic reticulum with an 88-kDa chaperonin which has been postulated to promote peptide binding and which may retain incompletely assembled class I molecules in the endoplasmic reticulum (14, 15).

The levels of immunoreactive T_4 in homozygous transgenic mice were significantly reduced compared with levels in heterozygous littermates, although not to a degree to account for death at 3 weeks. It has not been possible to assay the bioactive thyroid hormone triiodothyronine (T_3), produced by deiodination of T_4, to confirm that death was due to thyroid deficiency. In the rat, maturation of deiodinases in the liver and kidney occurs between 2 and 3 weeks of age, and interference with this process may lead to acute thyroid deficiency at a time of rapid growth and development (35). Hepatic nuclear T_3 receptors also mature at this time (13), and impaired T_3 receptor function may compound T_3 deficiency in early neonatal life. There is no reason, however, why the *H-2K^b* transgene in the thyroid would affect peripheral deiodinase activity or T_3 receptors. On the other hand, peripheral conversion to bioactive T_3 could be impaired if the T_4 produced by the transgenic thyroid, although immunoreactive, was abnormal as a substrate for deiodinases.

Our results may be relevant to a postulated direct, nonimmune role of MHC class I molecules in autoimmune disease (20). The overexpression of MHC molecules early in autoimmune disease tissues is presumed to be a response, initially, to agents such as viruses or toxins postulated to trigger autoimmunity and, later, to cytokines secreted by infiltrating mononuclear cells. Tissue destruction and/or aberrant antigen presentation by overexpressed MHC class I molecules could be a preautoimmune step that triggers macrophage activation and precedes lymphocytic infiltration. Following induction of autoimmunity, MHC class I molecules may then play a classical role to target cytotoxic T cells. The degree of overexpression of MHC class I molecules in cells infected with reovirus (7- to 8-fold) (9) or exposed to gamma interferon (10-fold) (6, 12), and in thyroid cells infected with simian virus 40 (4-fold on immunofluorescence scoring) (3), is similar to that in the homozygous transgenic mice (4-fold compared with nontransgenic mice). Although not quantitated, the level of MHC class I overexpression in human autoimmune thyroid disease and IDD (4, 17, 19) appears to approximate that seen in the RIP-*K^b* model (1) and in the thyroid transgenic model described in this report. The role of MHC class I expression in autoimmunity may, however, be more complex in light of a recent report that the expression of MHC class I is reduced in splenocytes from NOD mice and peripheral blood lymphocytes from humans with IDD (16), although these findings await confirmation.

The absence of a lymphocytic infiltrate in the thyroid and the low level of cytotoxicity of transgenic lymphocytes to H-2K^b target cells indicates tolerance to the allogeneic class I expressed extrathymically, analogous to the β-cell transgenic model (1, 2, 27). It is possible that T cells are peripherally tolerant (27) of the transgenic alloantigen, as demonstrated for the transgenically expressed lymphocytic choriomeningitis virus glycoprotein in β cells (29). On the other hand, PCR can detect transgene mRNA in the thymus of RIP-*K^b* mice (25), and most importantly, tolerance to allogeneic skin grafts is acquired following transplantation of the thymus from neonatal bm1 RIP-*K^b* mice but not nontransgenic littermates to thymectomized and irradiated, bone marrow-reconstituted bm1 mice (21). This finding implies that the tolerance is due to low-level thymic expression of the transgene, with subsequent clonal deletion or anergy. The ability of thymus grafts from TP-*H-2K^b* transgenic mice to confer tolerance is currently under investigation. The thyroid transgenic mouse is a practical model for studying tolerance because of the minimal thyroid abnormality, accessibility of thyroid tissue, and prolonged survival of heterozygous mice.

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