Thymic Nurse Cell Multicellular Complexes in HY-TCR Transgenic Mice Demonstrate Their Association with MHC Restriction

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This study examines thymic nurse cell (TNC) function during T-cell development. It has been suggested that TNCs function in the removal of nonfunctional and/or apoptotic thymocytes and do not participate in major histocompatibility complex restriction. We analyzed TNCs isolated from both normal C57BL/6 mice and C57BL/6 TgN (TCRHY) mice (HY-TCR transgenic mice). Using confocal microscopic analyses of TNCs isolated from C57BL/6 animals, we showed that 75%–78% of the enclosed thymocyte subset was viable, and 87%–90% of these cells expressed both CD4 and CD8. CD4 and CD8 also were expressed on TNC thymocytes isolated from both male and female HY-TCR transgenic mice. The transgenic female thymus was shown to have 17 times more TNCs per milligram of thymus than the transgenic male thymus. TNCs from HY-TCR transgenic females were 8–10 μm larger than transgenic male TNCs, and the female TNCs contained five times more thymocytes within intracytoplasmic vacuoles, with less than 4% apoptosis. However, more than 42% of the thymocytes within transgenic male TNCs were apoptotic. The large number and size of TNCs containing viable thymocytes in the female transgenic thymus suggest that TNC function is not limited to the removal of apoptotic thymocytes. We believe that the selective uptake of viable double-positive thymocytes by TNCs in C57BL/6 and HY-TCR transgenic female mice provides evidence that this interaction occurs during the process of major histocompatibility complex restriction. Exp Biol Med 232:780–788, 2007

Key words: thymic nurse cells; MHC restriction; thymocyte development; H-Y TCR Mice

Introduction

Wekerle and Ketelson discovered thymic nurse cells (TNCs) in mice in 1980 (1, 2). This unique multicellular complex was described initially as a keratin-expressing cell containing several thymocytes completely enclosed within specialized cytoplasmic vacuoles. The number of enclosed thymocytes ranges from 50 to 200. TNCs were shown to express both class I and class II major histocompatibility complex (MHC) antigens on their cell surfaces as well as on the surfaces of the vacuoles surrounding internalized thymocytes. Subsequent reports questioned the existence of an epithelial cell with the capacity to internalize developing thymocytes (3). However, following their initial discovery in mice, TNCs were isolated from the thymus of fish, frogs, chickens, sheep, pigs, rats, and humans (1, 2, 4–6). The demonstration of their persistence through so many species relieved much of the debate about the existence of TNCs, and at the same time demonstrated their apparent importance to the thymus and T-cell development.

The function of TNCs during T-cell development has been the focus of several investigations (1, 2, 7, 8). Since these cells form large and fragile multicellular complexes with thymocytes, analyses of *in vivo* isolates have yielded little information. Lysis of these complexes upon removal from the thymus has made it difficult to determine the level of expression of either the αβTCR or CD69. On the other hand, studies using clones of TNCs that maintain the ability to selectively internalize immature $CD4^+CD8^+\alpha\beta TCR^{lo}$ thymocytes *in vitro* have been exploited for the study of TNC function (9, 10). The population of thymocytes released from the TNC interaction contains both viable and apoptotic subsets. The cells that remained within intracytoplasmic vacuoles die through the process of programmed cell death and are destroyed through lysosomal activity (11). The percentage of release of viable thymocytes from TNCs was drastically reduced with the addition of antibodies against either class I or class II MHC antigens to cocultures (12). In addition, the TNC-rescued population

This work was supported by the National Science Foundation grant MCB-0412822, the National Institutes of Health–Research Centers in Minority Institutions (NIH-RCMI) grant 5G12RR03060, and NIH/SCORE grant SO6GM008168.

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Received December 8, 2006.
Accepted January 29, 2007.

1535-3702/07/2326-0780$15.00

matured from the $\alpha\beta TCR^{lo}CD69^{-}$ phenotype to $\alpha\beta TCR^{hi}CD69^{+}$-expressing cells in the presence of interleukin-1β (IL-1β; Ref. 13). This maturation shift has been reported to be an early step in positive selection (14). In addition, IL-1β was shown to be able to induce the presentation of self-antigen in cortical epithelial cells (15, 16). These results suggested that the TNC rescue of early triple-positive thymocytes from apoptosis was associated with an interaction between the TCR and the MHC.

To further investigate the role of TNCs in the process of MHC restriction, we used the HY-TCR transgenic mouse model, in which the entire complement of thymocytes exclusively expresses an αβTCR that recognizes the male-specific H-Y antigen. Virtually all HY-specific, αβTCR-bearing thymocytes are deleted from male thymi (negative selection), whereas positive selection of thymocytes recognizing the selective MHC background is significantly higher than normal in females (18, 19). This model system allowed us to examine the involvement of TNCs in the process of both negative and positive selection. In the studies reported here, confocal microscopy was used to show that TNCs isolated from C57BL/6 mice contained $CD4^{+}CD8^{+}$ thymocytes. Using this technology we were able to visualize each thymocyte in the complex. It was determined that 87%–90% of the thymocytes within the complex expressed both cell surface markers CD4 and CD8. Similar results were obtained using females from the HY-TCR transgenic mouse. The results from these experiments support the current concept that self-antigen presentation drives the selection process during thymic education. More specifically stated, thymocytes bearing self-antigen–specific αβTCR are deleted during MHC restriction in the male HY-TCR transgenic animal. The results of experiments obtained in this report revealed extensive differences between TNCs isolated from male HY-TCR transgenic mice *versus* those isolated from female transgenic animals. The female thymus was shown to have 17 times more TNCs per milligram of thymus than the male thymus. Female TNCs were 8–10 μm larger than male TNCs and contained five times more thymocytes within intracytoplasmic vacuoles. If TNCs only functioned in the removal of apoptotic thymocytes, it would be expected that relatively low numbers of TNCs would exist in the female HY-TCR transgenic thymic microenvironment, where few apoptotic thymocytes exist. Our finding that remarkably large numbers of TNCs are present in a microenvironment that is almost exclusive to positive selection suggests a function for TNCs in MHC restriction that is not limited to the removal of apoptotic thymocytes.

Materials and Methods

Mice. C57BL/6 mice (4–6 weeks old) used in these experiments were purchased from Jackson Laboratory (Bar Harbor, ME). HY-TCR transgenic mice (C57BL/6 TgN; TCRHY) were purchased from Taconic Animal Models (Germantown, NY). These animals express an αβTCR specific to the male restricted H-Y antigen.

Isolation of TNCs. The thymi of 4- to 6-week old mice were aseptically removed and mechanically dispersed in a mixture of 0.15% collagenase A (Roche Molecular Biochemicals, Indianapolis, IN) in 0.25% trypsin (GIBCO, Grand Island, NY). TNCs then were isolated using 1× g fetal calf serum gradient, as described by Wekerle and Ketelson (1, 2).

Tissue and Cell Staining. Mice were euthanized and were perfused with 10 ml of 4% paraformaldehyde, and the thymi were removed using aseptic techniques. Thymi were allowed to stabilize in 30% sucrose for 12 hrs before mounting on embedding molds (Polysciences Inc., Warrington, PA). Neg 50 embedding fluid (3 ml; Richard-Allen Scientific, Kalamazoo, MI) was added, and thymi were rapidly frozen using dry ice. Sections (6 μm) were obtained using a Micron Cryostat HM560 (Richard-Allen Scientific) and were mounted on Bond Rite slides (Richard-Allen Scientific). Sections were blocked for nonspecific binding by soaking for 1 hr in a blocking solution containing 0.1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) and 0.2% goat serum (Pierce, Rockford, IL) in phosphate-buffered saline (PBS). Sections were incubated for 1 hr in primary staining solution consisting of 2 μg/ml PH91 or anti-CDR1 (used as a positive control for thymic cortical epithelial cells) in blocking solution. After several washes in PBS, sections were incubated for 1 hr in a secondary staining solution (1:3000 dilution of anti-rat IgG 2a-FITC [fluorescein isothiocyanate] in blocking solution).

In preparation for staining, freshly isolated TNCs were placed on glass slides, air dried, and then fixed with 4% paraformaldehyde. Fixed cells were washed twice with PBS and then permeabilized by incubation for 10 mins in ice-cold acetone. Cells were again washed twice in PBS and then incubated for 45 mins at 4°C in a staining solution containing anti-CD4 biotin (Pharmingen, San Diego, CA) and anti-CD8 FITC (Pharmingen) antibodies at a concentration of 2 μg/ml in PBS. Cells were washed as previously described and then incubated for 45 mins at 4°C in a solution containing 1 μg/ml streptavidin–Alexa Fluor 488 (Pharmingen) in PBS. IgG 2a-FITC was used as a secondary antibody control. Preparations then were stained with diamidino-2-phenylindole (DAPI) to visualize thymocyte nuclei. Cells then were analyzed using a Zeiss LSM 510 confocal microscope (Zeiss, Thornwood, NY).

Terminal Deoxynucleotidyltransferase-Mediated dUTP-Biotin Nick End Labeling (TUNEL) Assay. Freshly isolated TNC complexes were fixed with 4% paraformaldehyde and then permeabilized in 0.1% Triton X-100 in PBS at 0°C for 2 mins. The *In Situ* Cell Death Kit, AP (Roche Diagnostics Corp., Indianapolis, IN) was used to detect apoptotic thymocytes, following the directions supplied by the manufacturer. After staining, cells were analyzed with a Zeiss fluorescence microscope. The percentage of TUNEL-positive, TNC-internalized thymo-

Table 1. Comparison of TNCs from Normal *Versus* HY-TCR Transgenic Mice[a]

Mouse strain	Average weight of thymus (mg)	No. of TNC complexes in thymus	Average size of TNC complexes (μm)	Average no. of TNC-internalized thymocytes
C57BL/6 female	59.8 ± 5.8	$6.82 \times 10^7 \pm 2.0 \times 10^6$	28.27 ± 2.9	12 ± 2
C57BL/6 male	40.8 ± 7.6****	$8.49 \times 10^7 \pm 6.7 \times 10^6$	28.44 ± 3.9	13 ± 2
HY-TCR transgenic female	35.7 ± 1.9*	$7.70 \times 10^8 \pm 2.5 \times 10^6$	31.55 ± 1.56	11 ± 1
HY-TCR transgenic male	15.6 ± 1.6**,***,****	$1.92 \times 10^7 \pm 5.0 \times 10^5$*,**,***	13.28 ± 0.81*,**,***	2 ± 0.1*,**,***

[a] The data shown represent the results (mean ± standard deviation) collected from three independent experiments with three animals per group ($n = 9$).
* $P < 0.01$ compared with C57BL/6 female; ** $P < 0.01$ compared with C57BL/6 male; *** $P < 0.01$ compared with HY-TCR female; **** $P < 0.05$ compared with C57BL/6 female. Statistical significance (Student *t*-test).

cytes was calculated by observing 100 TNCs from four different animals.

Results

Differences in TNC Populations Between the Sexes of HY-TCR Transgenic Mice. The thymi of HY-TCR transgenic mice were examined for differences in the size and number of TNC complexes present per thymic weight. The thymi of female HY-TCR transgenic mice contained an exponentially larger number of TNCs (Table 1 and Fig. 1A) than male HY-TCR transgenic mice (Table 1 and Fig. 1B). It was also noted that the average weight of the female HY-TCR transgenic mouse thymus was significantly larger, measuring approximately 35.7 mg (Table 1), than the male transgenic thymus, which weighed on average 15.6 mg. Although the female transgenic thymus was usually twice the weight of its male counterpart, both transgenic thymi were found to be significantly smaller than the average thymus size of C57BL/6 animals (Table 1). The numbers of TNCs isolated from the thymi of both female (Table 1 and Fig. 1A) and male (Table 1 and Fig. 1B) HY-TCR transgenic mice were compared to TNC numbers from the thymi of both sexes of the C57BL/6 strain (Table 1). These analyses revealed a much larger number of TNCs to be present in the female transgenic thymus *versus* control C57BL/6 animals (male or female). Female transgenic animals were found to produce 10–20 times more TNCs/mg thymus than C57BL/6 mice, and 17 times more than their male transgenic counterparts (Fig. 2A). As shown in Figure 1A, TNCs were found to be extremely abundant in the female transgenic thymus but were difficult to visualize at a magnification of ×100 in the male HY-TCR transgenic mouse (Fig. 1B). The average size of a TNC isolated from female transgenic animals was 32 μm in diameter, whereas transgenic male TNCs were 13.3 μm in diameter (Table 1 and Fig. 1C and D). Analyses of the number of thymocytes enclosed per TNC complex showed female transgenic TNCs to contain approximately the same number of thymocytes as those from C57BL/6 animals (Table 1). However, transgenic female TNCs generally contained five times more thymocytes than transgenic male TNCs. To emphasize these data further, when the ratio of internalized thymocytes to TNC diameter was considered, this ratio consistently measured 1:2 for C57BL/6 and for transgenic female animals, whereas the ratio of internalized thymocytes to TNC diameter for male TNCs was found to be 1:9.

TNCs isolated from male and female HY-TCR transgenic mice (Figs. 1E and F and 2B) also were analyzed using the TUNEL assay to determine the number of apoptotic thymocytes present in each complex. The levels of apoptotic thymocytes present in TNC complexes of transgenic animals were compared to those seen in normal C57BL/6 animals. It was observed that the average percentage of thymocyte apoptosis in TNCs isolated from C57BL/6 mice was approximately 20%, with no remarkable difference between the sexes in these animals. However, fewer than 4% of the thymocytes within TNCs isolated from female transgenic mice were found to be apoptotic, but 42% of the thymocytes within TNCs isolated from male HY-TCR transgenic mice were apoptotic (Figs. 1 and 2B).

Comparative Analysis of Wild-Type and HY-TCR Transgenic Thymic Cortices. PH91, a monoclonal antibody, was developed in our laboratory. PH91 exclusively identifies TNCs staining freshly isolated as well as cultured cells (19, 20). In addition, in thymic sections PH91 consistently stains portions of the cortical region. In the present study, we used PH91 and anti-CDR1, an established cortical epithelial marker, to determine the distribution of TNCs in the cortex of wild-type and transgenic mice (Fig. 3). In contrast to the thymi of C57BL/6 mice, we observed that PH91-stained TNCs were more extensive in the thymic cortex of female HY-TCR transgenic animals (Fig. 3G and H). It was also noted that unlike in transgenic females, fewer PH91-stained TNCs were detected in the male HY-TCR transgenic animal (Fig. 3K and L). Correspondingly, the cortex staining of HY-TCR transgenic males (Fig. 3I) is less extensive than that of wild-type animals and H-Y transgenic females. The results show that TNCs represent a *bona fide* subset of cortical epithelial cells.

Analyses of $CD4^+CD8^+$ Thymocytes in TNCs of HY-TCR Transgenic Mice. Our laboratory has previously reported that thymocytes internalized into TNCs isolated from C57BL/6 mice are at the $CD4^+CD8^+\alpha\beta TCR^{lo}$

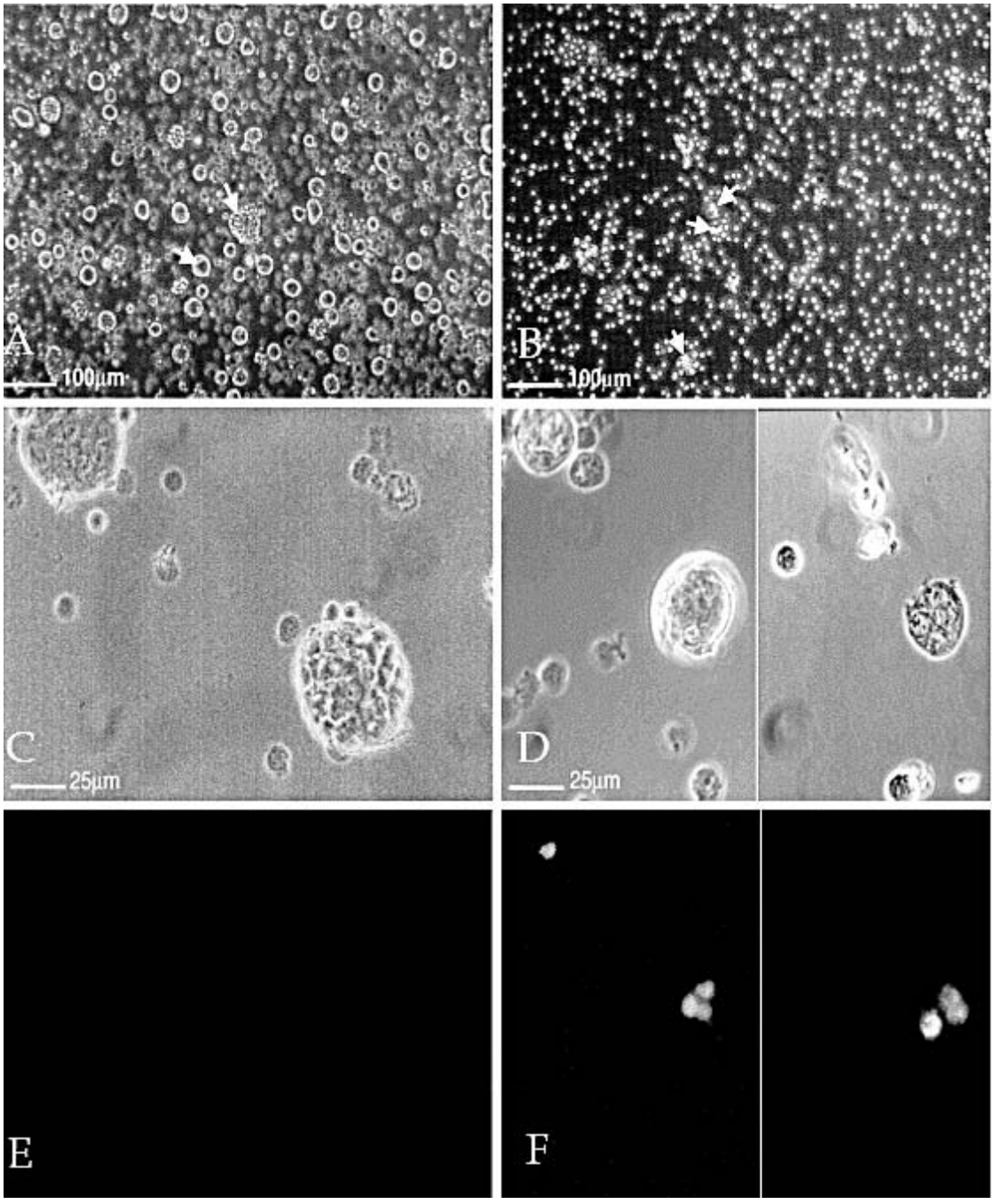

Figure 1. Comparative analyses of TNCs from HY-TCR transgenic mice. (A and C) Light micrograph studies of TNCs isolated from female HY-TCR transgenic mice. (B and D) Light micrograph studies of TNCs isolated from male HY-TCR transgenic mice. White arrows indicate TNCs. (E) TUNEL analyses of cells shown in panel C. (F) TUNEL analyses of cells shown in panel D. Magnifications: ×100 (A and B); ×630 (C–F).

developmental stage (9, 10). It has been proposed by other laboratories that thymocyte uptake by TNCs was restricted to nonfunctional and apoptotic thymocytes (21, 22). In order to determine the specificity of thymocyte uptake by TNCs, we used confocal microscopy to analyze CD4 and CD8 expression on the surface of internalized thymocytes. We did not include analyses of αβTCR expression in these studies, because previous work showed it to be expressed at an abnormally early stage of development in HY-TCR transgenic animals. Confocal microscopy enabled us to analyze each cell of the thymocyte population internalized into all TNCs examined. As shown in Figure 4, TNCs isolated from either C57BL/6 (Fig. 4A) or female HY-TCR transgenic mice (Fig. 4C) contained significantly large numbers of $CD4^{+}CD8^{+}$ thymocytes. A comparative analysis of DAPI-stained TNCs show that the internalized thymocytes in HY-TCR female animals are tightly packed compared with those in HY-TCR male animals (Fig. 4B and 4C). Using

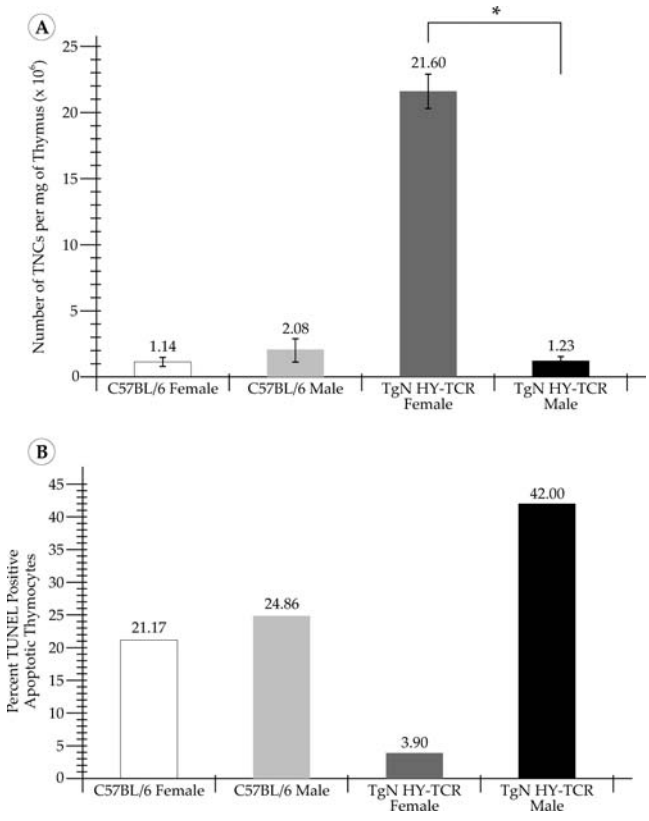

Figure 2. TNC number in thymus and apoptotic thymocyte population in TNCs of C57BL/6 and TgN HY-TCR mice. (A) Number of TNCs per milligram of thymic weight for C57BL/6 and TgN HY-TCR mice. *Difference between data is statistically significant (Student *t*-test, $P < 0.01$). (B) Percentage of TUNEL-positive thymocytes within TNCs of C57BL/6 and TgN HY-TCR mice. The data shown represent the results collected from one of three independent experiments. Three animals per group were used for each experiment.

confocal imaging at 1.0-μm intervals it was observed that these double-positive thymocytes were found throughout TNCs isolated from C57BL/6 mice (data not shown) or from transgenic females (Fig. 5A). Using quantitative analytical software, the percentage of double-positive thymocytes found throughout the TNCs of C57BL/6 or female HY-TCR transgenic animals was calculated to range from 87% to 90%. Similar examinations were conducted on TNCs isolated from the male HY-TCR transgenic thymi (Fig. 4B). The data collected showed that no thymocytes with a double-positive phenotype were present within TNCs of the male HY-TCR transgenic thymi (Fig. 5B). Instead, the cytoplasm of the TNC appeared to contain diffuse particles that costained with antibodies to both CD4 and CD8 (Fig. 4B). In addition, one of the three thymocyte nuclei contained in the TNC shown in Figure 4B (DAPI) is clearly fragmented. Several empty vacuoles also were observed within the TNC cytoplasm of male transgenic animals (Fig. 5B). These results are consistent with all TNCs examined in each of four independent experiments.

Discussion

We used the HY-TCR transgenic mouse model system to study the involvement of TNCs in MHC restriction. MHC restriction involves an interaction between the αβTCR on developing thymocytes and the self-peptides associated with MHC antigens on the cell surfaces of antigen-presenting cells, including epithelial cells, dendritic cells, and macrophages (24). During MHC restriction, developing thymocytes can be positively or negatively selected. Negative selection is facilitated through a high-affinity interaction between the αβTCR and the self-peptide in association with MHC antigens (23, 24). Thymocytes bearing αβTCRs that bind tightly to self-peptides in the context of MHC antigens are not allowed to mature and are selectively deleted because they are potentially autoreactive. Conversely, those thymocytes that produce αβTCRs that bind to self-peptides with low affinity are allowed to mature to the single-positive phenotype and are ultimately released from the thymus as functional T cells. The results reported in this study indicate that the multicellular complexes that define TNCs are associated with thymocytes participating in MHC restriction.

It has been proposed that the thymi of female HY-TCR transgenic animals produce a significantly larger than normal number of positively selected thymocytes because they lack the restrictive male-specific HY antigen (18, 25). Interestingly, our examination of the thymi from female transgenic mice showed significant differences in the TNC populations of these animals compared with male HY-TCR transgenic animals or C57BL/6 animals. There was an exponentially larger number of TNCs in female HY-TCR transgenic animals than in their male transgenic counterparts or in C57BL/6 mice (male or female; Table 1 and Fig. 1). To emphasize these differences further, it was noted that although the thymi of HY transgenic females were usually smaller than those found in normal C57BL/6 mice, they contained 10–20 times more TNCs (Table 1). More significantly, however, is that fewer than 4% of the TNC-internalized thymocyte population were found to be apoptotic in HY-TCR transgenic females. This was remarkably different from the 42% apoptotic thymocyte subset found within TNCs in transgenic males or the 25% found in C57BL/6 mice (Figs. 1 and 2B). These findings deviate from reports suggesting that TNCs function only in the removal of apoptotic thymocytes but are not involved in the MHC restriction process (22, 23). Such reports suggest that some of the enclosed apoptotic thymocytes are abnormal and may have been induced to die by mechanisms other than MHC restriction. If TNCs only functioned in apoptotic thymocyte clearance, very few complexes would have been detected in the thymi of female HY-TCR transgenic animals, where the numbers of apoptotic thymocytes were significantly low. Confirmation of these findings was obtained through analyses of TNCs isolated from HY-TCR transgenic male thymi. Virtually all HY-

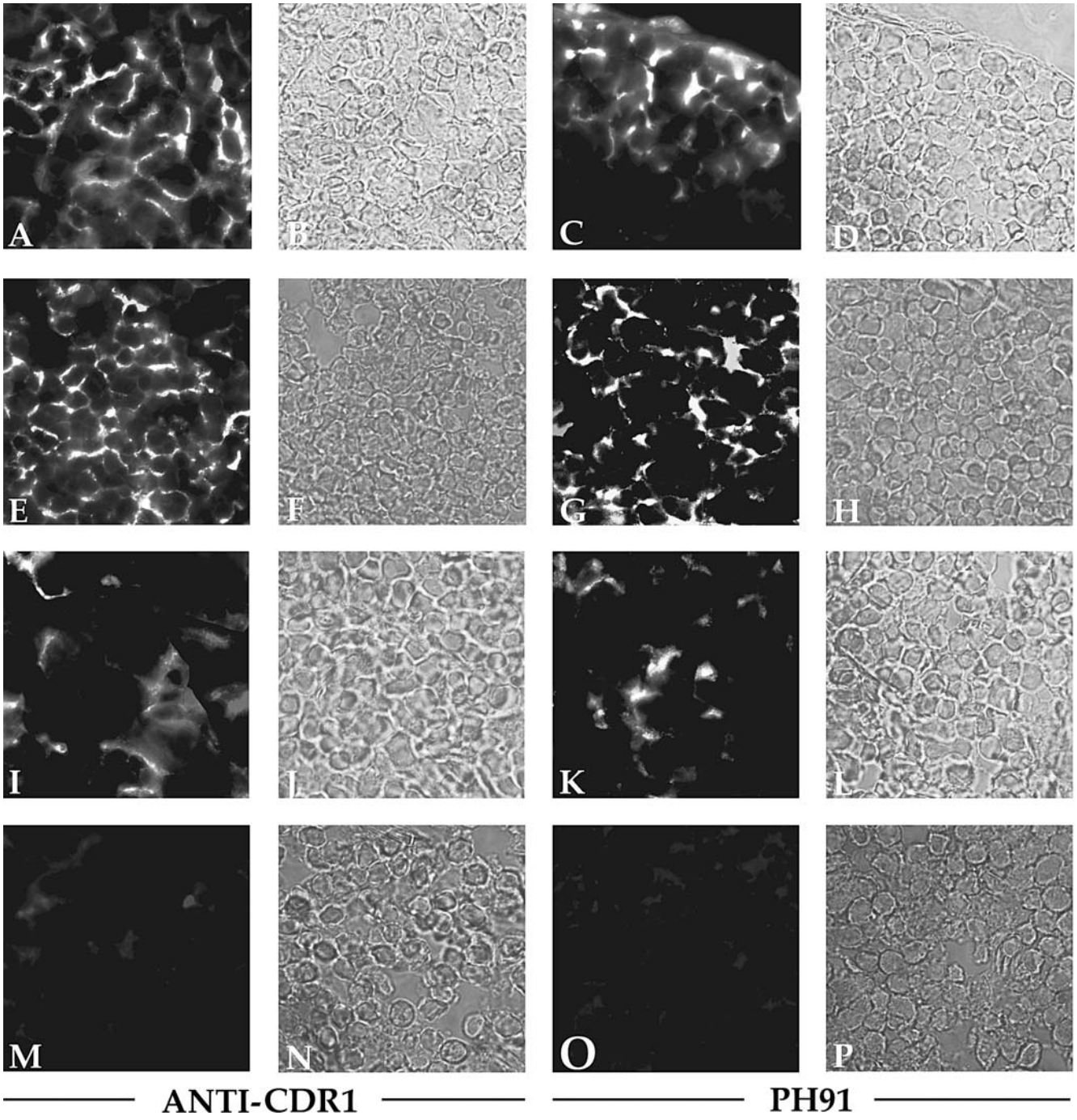

Figure 3. Identification of thymic epithelium in wild-type and HY-TCR transgenic mice. Thymic sections from C57BL/6 mice (A–D), female HY-TCR transgenic mice (E–H), and male HY-TCR transgenic mice (I–L) were prepared and stained with CDR1, a positive control for thymic cortical epithelium (first column), or PH91 antibody (third column) as described in Materials and Methods. The second and fourth columns are phase micrographs of the columns to the right. (M–P) Controls for which sections were incubated only with secondary antibody. Magnification: ×400. Color figure available in on-line version of journal.

specific αβTCR-bearing thymocytes are deleted from the thymus of these animals (17, 26). If TNCs function only in the removal of apoptotic thymocytes, one would expect to see significantly larger numbers of TNCs in the HY-TCR transgenic male thymus compared with the transgenic female thymus, corresponding to the larger number of apoptotic thymocytes reported to be present in the male (25). The data presented here question the validity of this surmise. Rather, these results suggest that TNC function may extend beyond the mere removal of apoptotic thymocytes.

Further examinations of the thymi of HY-TCR transgenic female mice revealed that TNCs penetrated deeper into the thymic cortex in these animals than in the C57BL/6

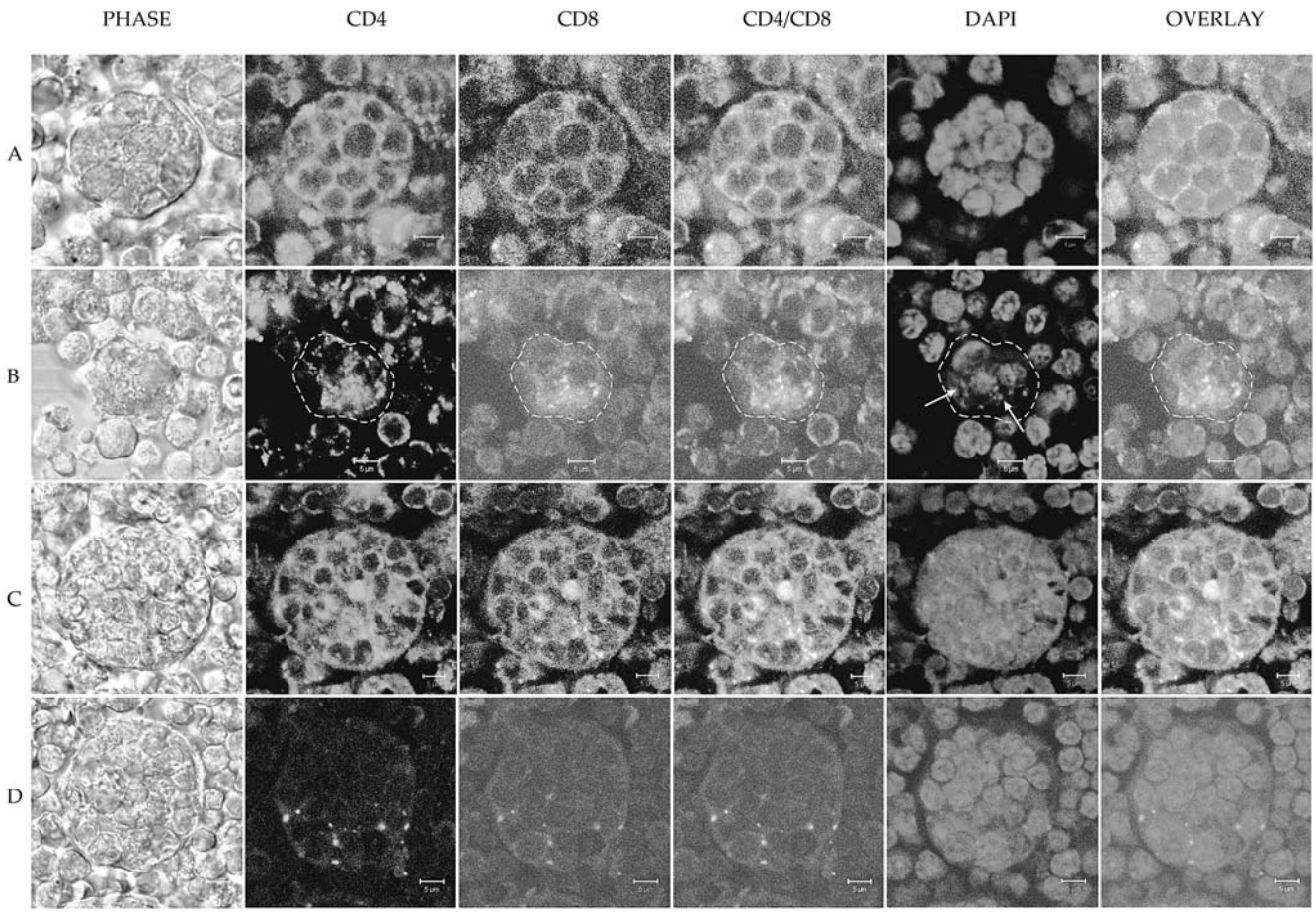

Figure 4. TNC-internalized thymocytes co-express CD4 and CD8. Confocal microscopic images of TNCs isolated from C57BL/6 (A), HY-TCR transgenic male (B), and HY-TCR transgenic female (C) mice. TNCs were stained with anti-CD4 (red) and anti-CD8 (green) antibodies as well as the nuclear-staining DAPI (blue), as described in Materials and Methods. Overlays of CD4 and CD8 are shown in the column labeled *CD4/CD8*. Overlays of CD4, CD8, and DAPI are shown in the column labeled *Overlay*. The first column shows phase contrast of the cells shown in rows A–D. Row D shows the results obtained when the TNC shown was stained with secondary antibody only. The images shown are representative of data collected from four independent sets of experiments ($n = 3$ for each group). Arrows indicate the presence of fragmented thymocyte nuclei. Each scale bar corresponds to 5 μm. Color figure is available in the on-line version of the journal.

thymus (Fig. 3). We suggest this may be due to the overabundance of TNCs found in the thymi of the HY transgenic female as opposed to C57BL/6 or HY-TCR transgenic male animals. In normal C57BL/6 male and female and HY-TCR female animals tested, TNCs were found to interact only with $CD4^+CD8^+$ thymocytes (Figs. 4 and 5). Analyses of the TNC-internalized thymocyte subset showed that approximately 87%–90% of the thymocyte subset found within the TNCs of C57BL/6 and HY-TCR transgenic female animals consistently costained with antibodies to CD4 and CD8. This double-positive thymocyte subset is reported to be participating in MHC restriction in the thymic cortex (9, 12, 27). These results suggest that thymocyte uptake by TNCs is very selective and involves the uptake of cells other than nonfunctional apoptotic thymocytes. This is exemplified by the observation that female HY-TCR transgenic animals internalize viable double-positive thymocytes with a significantly low level of apoptosis (Figs. 1 and 2B). On the other hand, very few TNCs were observed in the thymi of HY-TCR transgenic males, and thymocytes found within these TNCs were notably apoptotic (Fig. 1). Earlier work done by this laboratory has shown that apoptotic thymocytes within TNCs are degraded through TNC lysosomal activity (11). We suggest that the diffuse staining of the cytoplasm of TNCs isolated from male HY-TCR transgenic mice with antibodies to CD4 and CD8 (Figs. 4B and 5B) represents the degraded fragments of these apoptotic thymocytes. Our data show that 87%–90% of the internalized population expresses CD4 and CD8. We suggest that this difference represents the number of apoptotic thymocytes.

Van Ewijk *et al.* (26, 27), using immunohistologic techniques, showed the cortex of male HY-TCR transgenic mice to be abnormal compared with the thymic microenvironment of the female transgenic animal or C57BL/6 mouse. It was proposed that the viability of thymic epithelial cells requires the presence of a thymocyte-interactive population (28). If this is correct, our data suggest that the small number and size of TNCs found in the male HY-TCR transgenic thymus might result from its decreased number of

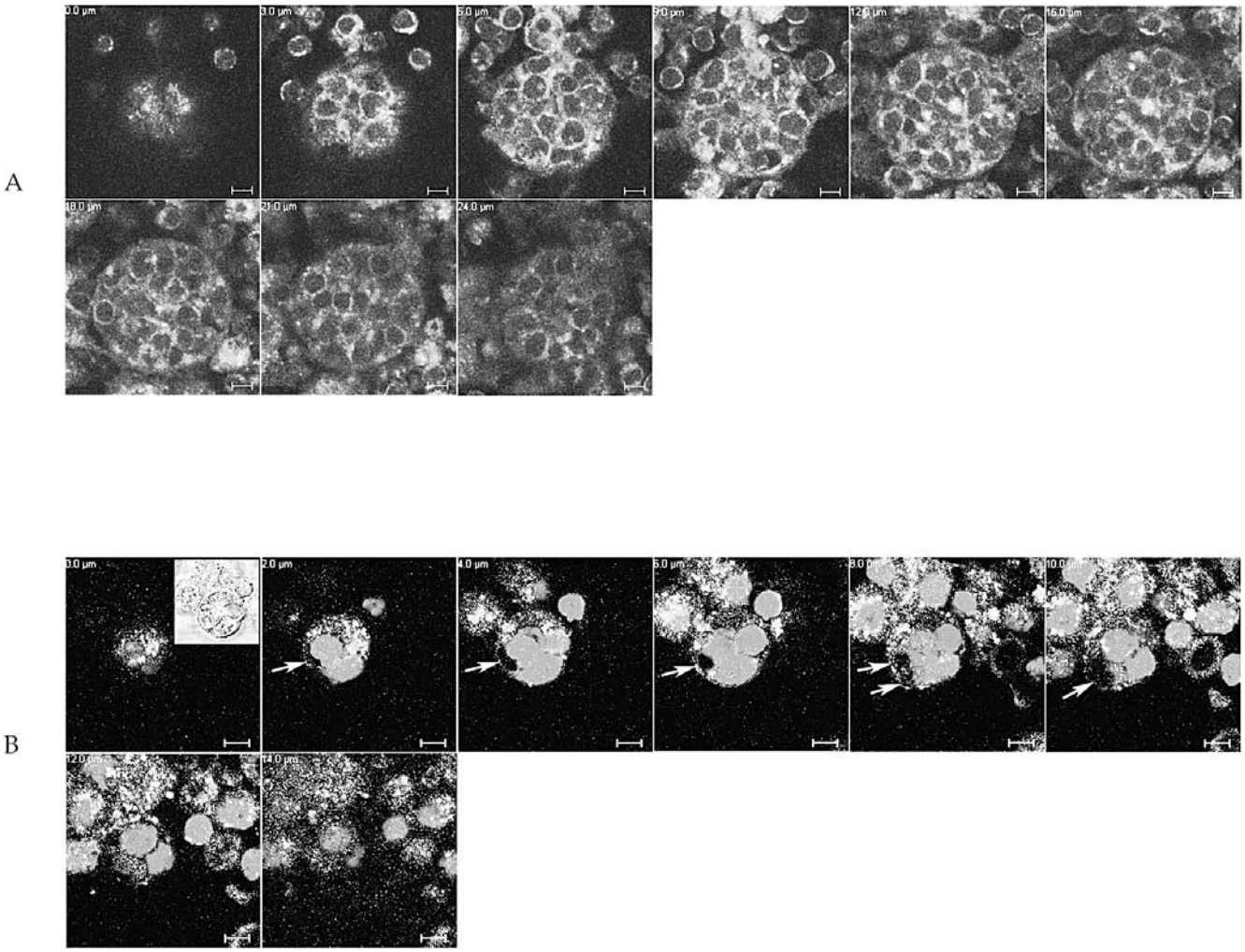

Figure 5. Analysis of $CD4^{+}CD8^{+}$ expression on TNC-internalized thymocyte population in HY-TCR mice. Confocal microscopic images were taken at 1.0-µm intervals through the TNCs shown in Fig. 4B and C to show colocalization of CD4 and CD8. TNC from female H-Y mouse shown at 3.0-µm intervals (A) and male H-Y mouse shown at 2.0-µm intervals (B). DAPI was included to show the locations of intact internalized thymocytes. Arrows show the location of an empty vacuole. The images shown are representative of data collected from four independent sets of experiments ($n = 3$ for each group). Inset shows the phase image of that TNC at 6 µm. Each scale bar corresponds to 5 µm. Color figure is available in on-line version of the journal.

thymocytes. The reduced number of both the double-positive thymocyte subsets and TNCs may contribute to the abnormal cortical morphology observed in the HY-TCR male transgenic animal (28).

Despite recent data suggesting that TNCs merely function in the removal of nonfunctional and apoptotic thymocytes, the data reported here show TNCs to be associated with viable positively selected thymocytes that almost exclusively populate the female HY-TCR transgenic thymus. We submit that if TNCs function only in the removal of apoptotic thymocytes, a significantly larger number of TNCs would have been found in the transgenic male animal. We also propose that because thymocyte uptake is restricted to the double-positive subset, the interaction between TNCs and their enclosed thymocyte population has a function during MHC restriction.

Further, the function of TNCs has been studied in diseased animals. Evaluations of several autoimmune animal models, NZB-Bln, MRL/MP-Faslpr (MRL/*lpr*), and C3H/HeJ-Fasgld (C3H/*gld*) mice, show a 30%–50% reduction in the number of TNCs (29–31). It has been proposed that reduction of these specialized epithelial cells may play a role in the development of the autoimmune phenotype. In this report we have postulated that TNCs participate in both positive and negative selection during the process of MHC restriction. The removal or absence of a large number of TNCs from the thymic microenvironment would increase the possibility of miseducated thymocytes. As a result, potentially autoreactive thymocytes can continue the maturation process, emigrate from the thymus, and populate peripheral lymphoid centers. Further, a reduction in the quantity of TNCs may also affect the clearance of apoptotic thymocytes. We have previously shown that TNCs are involved in the degradation of TNC-induced apoptotic cells (20, 32, 33),

a function that was previously assigned exclusively to thymic macrophages (34). Thus, perturbations in TNC function could lead to the development of systemic autoimmunity. Taken together, TNCs are central to the development of thymocytes and play an essential role in the production of self-tolerant T cells.

We would like to thank Mr. Moazzam Ali Brohi for his technical expertise in the production of figures for this paper, and Dr. Masako Osada, Mr. Junchen Li, and Mr. Andrew Blake for providing us with the monoclonal antibody PH91. We would like to give special thanks to Mr. Douey Wright, Ms. Mishanta Reyes, and Mr. Zacharia Olushoga for their invaluable assistance in the completion of many small projects for this paper. We would also like to thank Mr. Daniel Fimiarz for his assistance with confocal microscopy. We are also grateful to Malikqua Lancaster for the statistical analysis.

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