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Thymocytes Express the *golli* Products of the Myelin Basic Protein Gene and Levels of Expression Are Stage Dependent

Ji-Ming Feng,* Irene M. Givogri,* Ernesto R. Bongarzone,* Celia Campagnoni,* Erin Jacobs,* Vance W. Handley,* Vilma Schonmann,* and Anthony T. Campagnoni2*†

The *golli* products of the myelin basic protein gene have been shown to be expressed in mouse thymus and brain. The full repertoire of thymic cell types expressing *golli* products has not yet been determined, although immunoreactivity has been found in some macrophages. We have analyzed the cellular expression of *golli* mRNAs and proteins in the thymus. The results showed that MTSS1* cortical/MTS10* medullary epithelial cells and NLDC145* dendritic cells did not express *golli*, while some macrophages did exhibit strong immunoreactivity. *Golli* mRNAs were not detected in macrophages by in situ hybridization. Thymocytes expressed significant levels of *golli* mRNAs and proteins by in situ hybridization and immunohistochemistry. Interestingly, *golli* immunoreactivity varied with thymocyte stage of differentiation. For example, CD4+CD8+ expressed significant levels of *golli* protein; P, postnatal day; RPA, RNase protection assay; RT, room temperature; SP, single positive; TSA-FISH, tyramide signal amplification for fluorescence in situ hybridization.

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2 Developmental Biology Group, Neuropsychiatric Institute, and 1 Brain Research Institute, University of California Medical School, Los Angeles, CA 90024

*Address correspondence and reprint requests to Dr. Anthony T. Campagnoni, University of California Medical School, Neuropsychiatric Institute, Room 47-448, 760 Westwood Plaza, Los Angeles, CA 90024-17519.

E xperimental allergic encephalomyelitis (EAE) has served as a model of autoimmune disease (1); and the myelin basic proteins (MBPs) were the first proteins identified in the CNS to induce this disease in laboratory animals (2–5). For many years, it was assumed that the reason MBPs induced the disease was because the immune system was protected from exposure to these proteins by the blood-brain barrier.

In recent years, it has been found that the MBP gene encodes two families of products containing classic MBP epitopes. In addition to the classic MBPs found in the myelin sheath and expressed by myelin-forming cells, a second set of products, called *golli* proteins and mRNAs, is produced by the gene in the immune and nervous systems of mice, rats, and humans (6, 7). The MBP gene, the two families of products it expresses, and the common MBP sequences in the *golli* and classic MBPs are shown in Fig. 1. The *golli* products differ from the classic MBP proteins through the presence of a 133-aa sequence unique to the *golli* proteins, encoded by several exons upstream of the classic MBP transcription start site. These unique regions of the *golli* proteins and mRNA gene products have permitted us to generate specific Abs and specific probes (8) for the analysis of cellular expression of *golli* products at the protein and mRNA level. The presence of *golli* products in the early developing thymus in mouse and human (9, 10) suggests that lack of exposure of the immune system to MBP-related products cannot completely explain the autoimmune activity of the MBPs. Studies have shown that MBP epitopes found within the context of *golli* proteins can be recognized by T cell lines specific for encephalitogenic epitopes of classic MBP (11), which can induce severe EAE in mice. It has also been reported that *golli*-specific peptides alone can induce a mild autoimmune disease in the rat (12). Previous work has shown that *golli* mRNA expression increases during the relapsing phases of EAE, which has led to the proposition of an alternative model for epitope spreading during the course of EAE based upon endogenous expression of *golli* in lymphoid tissue (13).

Several lines of evidence indicate that *golli* proteins (or epitopes) can be found within some macrophages in the thymus and lymph nodes of mice and humans and that they are capable of presenting MBP epitopes derived from *golli* on their surface (13, 14). However, the expression of *golli* products in other cells in the thymus could not be excluded because of the high autofluorescence of the tissue (9). Thus, we undertook a more detailed examination of *golli* protein and mRNA expression in the thymus to identify all the cell types expressing these products of the gene. Such a study is an important component of determining the mechanisms underlying the induction of tolerance and the autoimmune response to MBPs in EAE. While the results confirmed the presence of immunoreactivity in some thymic macrophages, we were surprised to find that the majority of *golli* expression occurs in thymocytes, that this expression changes with the state of thymocyte maturation, and that there is little expression of *golli* in epithelial or dendritic cells.
Materials and Methods

Antibodies

The rabbit polyclonal anti-\textit{golli} Ab was generated against a recombinant peptide corresponding to the 133-aa specific to the \textit{golli} proteins; the antisera were affinity purified with the \textit{golli} peptide and characterized, as described previously (8). The other primary Abs used were: CD4 (L3T4) FITC, CD8a (Ly-2) PE, MTS5 (rat IgM), and MTS10 (rat IgM) mAbs (PharMingen, San Diego, CA); NLDC-145 (rat IgG2a; Serotec, Oxford, U.K.); and F4/80 (rat IgG2b; Caltag, South San Francisco, CA).

Tissue preparation for immunohistochemistry and in situ hybridization

BALB/cByJ mice bred in our animal colony from stock obtained from The Jackson Laboratory (Bar Harbor, ME) were used for analysis. Thymuses were removed from the mouse at postnatal day 6, embedded in OCT medium (Tissue-Tek, Sakura Finetek, Torrance, CA), and stored at -20°C until use. Tissue was sectioned at 2–5 μm, mounted on Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA), dried briefly at room temperature (RT), fixed in pure acetone at RT for 20 min, dried again in air, and stored at 4°C.

Ag detection by immunohistochemistry

The slides were hydrated in PBS for 15 min, and incubated in 1% BSA in PBS for 30 min. Sections were then incubated with primary Ab mixtures in 0.2% BSA/0.05% sodium azide (Ab dilution buffer) in PBS for 45 min at room temperature in a humid chamber. Following three washes (10 min each) with 0.05% Tween 20 in PBS, the sections were incubated for 45 min at RT with fluorescence-labeled secondary cocktails. The slides were then washed three times (10 min each) and mounted in 75% glycerol in PBS (pH 9.5). Fluorescence images were obtained using an excitation wavelength of 495 nm. Excitation wavelengths of 488 and 550 nm were used for PE and tetramethylrhodamine isothiocyanate red fluorescence, respectively. For double labeling, the primary Ab cocktails consisted of the following combinations: \textit{golli} Ab (1:500) + MTS5 (1:200) MTS5 (1:100), F4/80 (1:50), or NLDC-145 (1:50), respectively. The secondary Ab cocktails consisted of the anti-rabbit IgG tetramethylrhodamine isothiocyanate (Sigma, St. Louis, MO; 1:200) + anti-rat Ig light chain FITC (Sigma; 1:200).

Isolation of thymocytes

Cells were dispersed from the thymus tissue by extrusion through a stainless steel 200 mesh into PBS. After 2-h culture in the plastic dishes to remove the possible contaminated adherent stromal cells, the nonadherent thymocytes were collected by centrifugation. Following three washes in PBS, the thymocytes were subjected to FACS analysis or RNA extraction. The thymocyte preparations were routinely 99% pure, as determined by FACS analysis.

![Diagrammatic representation of the \textit{golli}-MBP gene illustrating the two families of MBP-related products expressed from the gene. \textit{golli} products are expressed from the first transcription start site of the gene, and classic MBPs are expressed from the two downstream promoters. All \textit{golli} proteins contain a 133-aa sequence (open box) at their N termini, and the two major \textit{golli} products contain MBP sequences in the C-terminal portions of the molecules. The MBP sequences (hatched boxes) found in each of the major \textit{golli} proteins are shown. The polyclonal Ab used to detect \textit{golli} protein expression in the thymus was prepared against the 133-aa \textit{golli}-specific domain, as published previously (10) and does not cross-react with MBPs.](http://www.jimmunol.org/)

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**FIGURE 1.** Diagrammatic representation of the \textit{golli}-MBP gene illustrating the two families of MBP-related products expressed from the gene. \textit{golli} products are expressed from the first transcription start site of the gene, and classic MBPs are expressed from the two downstream promoters. All \textit{golli} proteins contain a 133-aa sequence (open box) at their N termini, and the two major \textit{golli} products contain MBP sequences in the C-terminal portions of the molecules. The MBP sequences (hatched boxes) found in each of the major \textit{golli} proteins are shown. The polyclonal Ab used to detect \textit{golli} protein expression in the thymus was prepared against the 133-aa \textit{golli}-specific domain, as published previously (10) and does not cross-react with MBPs.
Triple labeling of thymocytes for FACS analysis and confocal microscopy

Approximately $5 \times 10^5$ to $1 \times 10^6$ freshly isolated thymocytes were incubated for 45 min at 4°C with CD4 FITC (0.5 μl) and CD8 PE (0.5 μl) in 200 μl Ab dilution buffer, washed with PBS twice, suspended, and fixed in 2% paraformaldehyde in PBS. After overnight fixation, the stained thymocytes were incubated with golli polyclonal Ab (45 min at 4°C). Following three washes, cells were incubated for 45 min at 4°C with Cy-5-conjugated secondary Ab (anti-rabbit IgG, 1:400; Jackson ImmunoResearch, West Grove, PA). After three washes, the cells were subjected to FACS analysis on FACS Calibur (Becton Dickinson, San Jose, CA) or were examined by confocal microscopy. All results displayed in FACS analysis are representatives of at least three independent experiments. An emission wavelength of 650 nm was used to examine Cy-5 fluorescence in the confocal microscope, but the fluorescence image was converted into a blue pseudo color.

Extraction of RNA and RNase protection assays (RPA)

**RNA extraction.** Total RNA was extracted from isolated thymocytes, normal thymus, and 20-h dexamethasone (DEX)-injected thymus (i.p. 50 mg/kg weight) using the Trizol procedure (Life Technologies, Gaithersburg, MD).

**Riboprobe.** The golli antiserum [3P]UTP riboprobes specific to exons 2, 3, and 5A (called G19) were synthesized from linearized plasmids with T7 polymerase.

**RPA assays.** RPA were performed with a commercially available kit from Ambion (Austin, TX). For RNase protection experiments, 10 μg total RNA was hybridized to the probe at 42°C. Samples were electrophoresed in a 6% polyacrylamide/8 M urea gel, and then subjected to autoradiography.

**Tyramide signal amplification for fluorescence in situ hybridization (TSA-FISH)**

After treatment with 0.2 M HCl for 10 min, sections were prehybridized for 30 min at 37°C in a solution containing 50% formamide, 750 mM NaCl, 0.1% SDS, 50 mM PIPES, pH 6.9, 0.2% Ficoll, 0.2% BSA, 0.2% polyvinylpyrrolidone, and 0.5 mg/ml ssDNA. Sections were then hybridized overnight at 45°C in prehybridization solution containing 1.5 ng/μl digoxigenin-labeled antisense golli cRNA probe (G19). After hybridization, sections were washed in RNase buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 M NaCl) for 30 min at 37°C, and then digested for 30 min at 37°C in RNase buffer containing 20 μg/ml RNase. Finally, sections were washed in RNase buffer without RNase (30 min, 37°C), 0.5× SSC (1 h, 60°C). The remaining steps were the same as described for immunohistochemistry. Briefly, sections were treated with 3% H2O2 in methanol for 15 min to quench the endogenous peroxidase, and then incubated with the anti-digoxigenin Ab conjugated with peroxidase (RT, 1 h). After a series of washes in TNT buffer, sections were incubated in the DNA probe. The sections were then digested in RNase buffer for 30 min at 37°C, and then digested for 30 min at 37°C in RNase buffer containing 20 μg/ml RNase. Finally, sections were washed in RNase buffer without RNase (30 min, 37°C), 0.5× SSC (1 h, 60°C). The remaining steps were the same as described for immunohistochemistry. Briefly, sections were treated with 3% H2O2 in methanol for 15 min to quench the endogenous peroxidase, and then incubated with the anti-digoxigenin Ab conjugated with peroxidase (RT, 1 h). After a series of washes in TNT buffer, sections were incubated in the DNA probe.

**Results**

**Distribution of golli immunoreactivity in thymic stromal cells**

Because of the role that stromal cells play in thymic selection, we were interested in determining the extent of golli expression in these cells by double immunofluorescence with anti-golli and known markers of stromal cells. Because autofluorescence increases markedly in the thymus with age, we performed the study on BALB/c (C57) mouse thymus (P6).

Fig. 2 shows thymic sections stained for golli immunoreactivity in red and several markers of stromal cells in green. F4/80 was used to mark thymic macrophages (15, 16). Occasionally, some F4/80+ macrophages within the medulla showed strong golli immunoreactivity, an example of which is shown in the inset, upper right of Fig. 2A, although most of the F4/80+ macrophages were golli negative (Fig. 2A). MTS10 and MTS5 were used to identify medullary (Fig. 2B) and cortical (Fig. 2C) epithelial cells, respectively (17); and NLDC-145 was used to mark dendritic cells and some cortical epithelial cells (Fig. 2D) (18–20). None of these dendritic or epithelial cells exhibited detectable levels of golli immunoreactivity.

**Thymocytes express golli proteins**

As Fig. 2, A–D indicates, many small round cells with prominent nuclei showed relatively low golli immunoreactivity. The location and morphology of these cells suggested they were thymocytes. To

![FIGURE 2.](http://www.jimmunol.org/)

Double-label immunofluorescence studies with golli (red fluorescence) and several stromal cell markers (green fluorescence). A, F4/80 was used to identify macrophages. Most F4/80+ macrophages did not exhibit detectable golli immunofluorescence. However, a subset of macrophages exhibited intense expression of golli, an example of which is shown in the inset in A. B, MTS10 Ab was used to visualize medullary epithelial cells. A section was chosen illustrating the border between the medulla and cortex, as shown. No detectable golli staining was seen in MTS10+ cells. C, Cortical epithelial cells, identified by MTS5 staining, did not express detectable golli proteins. D, No detectable golli immunoreactivity was associated with NLDC-145+ dendritic cells. Magnification in A and B, ×400; magnification in C and D, ×630.

![FIGURE 3.](http://www.jimmunol.org/)

Golli immunoreactivity is exhibited within isolated thymocytes, but not on their surface. A. Isolated thymocytes exhibit golli immunoreactivity after 1-h fixation in 4% paraformaldehyde, which permeabilizes the cells. The larger, blastlike thymocytes (indicated by arrows) showed stronger golli staining than smaller, more mature cells. C, No significant golli staining was observed in live, unfixed cells, indicating that the golli proteins are not located on the cell surface. B and D, Nomarski images for A and C, respectively. Magnification, ×400.
verify this, thymocytes were isolated and immunostained for golli proteins. Interestingly, only cells fixed and permeabilized were stained by the golli Ab (Fig. 3, A and B). Live staining of thymocytes showed no immunoreactivity for golli proteins (Fig. 3, C and D). This suggested that unlike surface markers such as CD4, CD8a, or CD3e, golli proteins were localized inside the thymocytes, and not on their surface, consistent with their localization in neural cells. Also, the level of intensity of golli staining varied widely among individual, isolated thymocytes. Larger thymocytes with blastocytic features (arrows, Fig. 3A) tended to exhibit more robust golli staining than smaller, apparently more mature, cells.

Changes in golli expression in developing thymocytes

Developing thymocytes can be divided into four populations, according to their expression of CD4 and CD8 surface markers. The least differentiated thymocytes are CD4−CD8− double-negative (DN) cells. They then transition to a CD4+CD8− double-positive (DP) stage and mature into either CD4+ or CD8+ single-positive (SP) populations (21, 22). Colocalization of golli in thymocytes at different developmental stages was performed by triple-label immunofluorescence and confocal microscopy (Fig. 4). The majority of isolated cells were DP thymocytes, staining for both CD4 and CD8 surface markers with varying intensity. Fewer cells in the population were SP or DN thymocytes. Almost all the thymocytes exhibited golli staining, but they seemed to distribute into two populations based upon staining levels, i.e., high expressers (gollihigh) and low expressers (gollilow). It seemed that the gollihigh cells colocalized primarily with DP thymocytes, but gollihigh cells colocalized primarily with SP (not shown in Fig. 4) and DN thymocytes. In DP thymocytes, there also seemed to be a correlation

FIGURE 4. Confocal images of thymocytes illustrating single, double, and triple labeling for golli (blue), CD4 (green), and CD8 (red). Four panels showing a field of cells individually labeled for golli, CD4, and CD8 as well as combined are shown. All DN cells and transitional DP cells (CD4low−CD8+ and CD4+CD8low) showed strong staining of golli. Most DP cells showed varying levels of staining, from golli negative to strong positive staining. The different types of labeled cells are shown in different colors: gollihigh DP, white; gollihigh DN, blue; gollilow DP, green; CD4low DP, orange; CD8low DP, pink. For ease of comparison among the panels, arrows and asterisks of different colors are used to indicate positions of corresponding cells. Magnification, ×400.
of levels of golli expression with whether the staining of CD4 or CD8 predominated. For example, the transitional CD4\textsuperscript{low}CD8\textsuperscript{+} and CD4\textsuperscript{+}CD8\textsuperscript{low} DP cells exhibited golli\textsuperscript{high} staining, but CD4\textsuperscript{+}CD8\textsuperscript{-} DP cells showed variable staining, from golli\textsuperscript{null} to golli\textsuperscript{high}. We wished to explore further the possibility that golli expression varied with the stage of thymocyte development and, since the microscopic technique was not quantitative, we turned to FACS analysis.

Triple-color FACS analysis with gating on the different populations was employed to further examine golli expression in thymocytes. The results (shown in Fig. 5, A–C) indicated that the percentage of different populations in isolated thymocytes were: DN, \( \sim 2\% \); DP, \( \sim 87\% \); CD4\textsuperscript{+} SP, \( \sim 7\% \); and CD8\textsuperscript{-} SP, \( \sim 4\% \). After gating, about 98\% DN, 88\% DP, 96\% CD4\textsuperscript{+} SP, and 97\% CD8\textsuperscript{-} SP thymocytes were golli\textsuperscript{+}. The mean values of golli fluorescence intensity in the populations were: DN (172 \pm 4) > CD8\textsuperscript{-} SP (140 \pm 3) \geq CD4\textsuperscript{+} SP (132 \pm 2) > DP (84 \pm 5). Thus, the least mature, DN cells expressed the highest levels of golli. Upon further differentiation into DP cells, golli expression declined dramatically (almost 2-fold). Further differentiation into SP cells resulted in an increase in golli expression again, although not to the levels found in DN cells.

**Thymocytes express golli in both cytoplasm and nuclei**

In the nervous system, golli proteins have been localized to both the nucleus and the cytoplasm, and in certain populations of neurons there occurs a shift in subcellular localization during development and migration. For this reason, we wished to determine the localization of golli proteins in thymocytes. Conventional microcopy clearly showed cytoplasmic labeling and suggested punctate labeling of golli in thymocyte nuclei (e.g., see Fig. 2, C and D), but to confirm this, sections were examined by confocal microscopy. Since \( \sim 98\% \) of thymocytes (including CD4\textsuperscript{+}CD8\textsuperscript{-} DP and CD4\textsuperscript{+} SP) express CD4 molecules on the surface, we used CD4 to mark the location of thymocytes in tissue sections. The optical sectioning images through the tissue clearly demonstrated the presence of golli immunoreactivity in the nuclei of thymocytes (Fig. 6A). In agreement with the cell-sorting data, the thymocyte population in the subcapsular zone (i.e., DN cells) exhibited stronger staining than the DP thymocytes in deeper regions of the cortex. The subcapsular region contains primarily immature DN cells. Immaturity and cell division are indicated by the presence of many mitotic figures and increased cell size of thymocytes in this zone revealed by staining with cresyl violet (arrows, Fig. 6B).

**Thymocytes express golli mRNAs**

Further proof of the expression of the golli-MBP gene in thymocytes was obtained by in situ hybridization of tissue sections using the TSA-FISH technique. A digoxigenin-labeled cRNA probe specific for golli exons 2, 3, and 5a was prepared and used to detect golli mRNAs in situ. Hybridization signal was clearly evident throughout thymocyte populations in the tissue. Again, high intensity of expression was noted in the subcapsular region, presumably arising from DN thymocytes (Fig. 7A–C).

Additional proof of golli expression in thymocytes and the large contribution of thymocyte expression to golli expression in the thymus was demonstrated by RPA experiments. As shown in Fig. 8A, high levels of golli mRNAs could be seen in both thymus and brain at both P6 and P24. Surprisingly, the levels of golli mRNAs were very similar in the two tissues at these ages, indicating proportionally high expression of this myelin protein gene product in the thymus. Low levels of golli mRNA expression were evident in P6 liver, which disappeared by P24, consistent with the decline in hemopoietic activity in this tissue during early postnatal development in the mouse. Another experiment is shown in Fig. 8B. Isolated, pure thymocytes expressed high levels of golli mRNAs, comparable with levels in the intact P6 thymus, and consistent with thymocytes as a major source of golli expression in this tissue. Further evidence of this was obtained by injecting P24 mice with DEX, known to severely deplete the thymus of thymocytes. Twenty hours after injection with DEX, RNA was isolated and a RPA analysis performed and compared with RNA from a PBS-injected control. The results showed a substantial decrease in the levels of golli mRNAs in the thymus, consistent with the loss of thymocytes brought about through the DEX injection (Fig. 8B).

**Discussion**

Golli expression occurs primarily in mouse thymocytes

Thymic cells can be divided into two populations, one of which is T lymphoid derived and represents \( \sim 95\% \) of the total cells in the thymus. This population includes T lineage cells at various stages of differentiation, located throughout the entire thymic parenchyma. The other population consists of non-T lymphoid cells, also called stromal cells, which include many epithelial cells that form a network extending from the cortex to the medulla, bone marrow-derived hemopoetic cells including macrophages and dendritic cells, and mesenchymal cells associated with connective tissue coursing throughout the thymus (23).

The results of this study indicate that thymocytes are the primary cell type to express golli in the thymus. Expression of golli mRNAs and proteins in thymocytes was established by several
means, including direct double- and triple-label immunofluorescent analysis on tissue sections and isolated thymocytes using cell-specific markers, FACS analysis, and RNase protection analyses of thymuses from normal and DEX-treated animals. In mice injected with DEX, there is a massive death of thymocytes and an increased proliferation of thymic macrophages. Our results showed a substantial reduction in golli mRNA in the DEX-treated thymus, consistent with a primary localization of these mRNAs in thymocytes. There was no increase in golli immunoreactivity in thymic macrophages as a consequence of DEX treatment (data not shown).

Golli expression does not occur in stromal cells, except for a few macrophages

In contrast with the immunofluorescent results indicating pervasive expression of golli in thymocytes, there was essentially no detectable immunofluorescence in stromal cells (i.e., MTS5+, NLDC-145+, and most F4/80+ cells), with the sole exception of some F4/80+ macrophages. Using TSA-FISH analysis, we were unable to detect significant expression of golli mRNAs in thymic macrophages. The presence of golli immunoreactivity in these cells could be due to the phagocytosis of apoptotic thymocytes or to instability of golli mRNAs in thymic macrophages compared with thymocytes.

The lack of golli expression in thymic stromal cells may explain inefficient central tolerance to MBP autoreactive T cells

It has been established that CD4+Th cells reactive to MBP mediate EAE, an animal model for some aspects of multiple sclerosis (24). The presence of autoreactive T cells specific to MBPs in healthy individuals makes it possible that these autoreactive T cells are normal components of the T cell repertoire, and suggests that in the thymus there must be inefficient negative selection for T cells autoreactive to MBPs (25).

There is substantial evidence, including this study, to indicate that products of MBP gene are expressed in the thymus, but it is not clear why the escape of autoreactive T cells specific for MBP occurs in the thymus. An affinity hypothesis has been put forward based on the fact that the dominance of encephalitogenic epitopes varies among strains depending upon the MHC background of the strains (26). For example, in the SJL(H-2s) strain, the 83-102 sequence is the immunodominant region, while 1-11 is the subdominant region; but in the PL and B10.PL (H-2u) strains, the immunodominant region is aa 1-11. In these instances, inefficient

FIGURE 6. CD4+CD8+ DN cells exhibit strong expression of golli proteins, particularly within thymocyte nuclei in vivo. A, A confocal image through a section of a P6 thymus illustrates the localization of golli immunoreactivity (red) in thymocyte nuclei. The section, which is costained for CD4, also illustrates that the DN thymocytes situated in the subcapsular region exhibit stronger staining than CD4+ thymocytes located deeper in the cortex. B, A neighboring section, stained with cresyl violet, shows many mitotic cells (arrows) of larger size and with visible chromosomes characteristic of dividing, immature cells in the subcapsular region. The double-headed arrows delineate the subcapsular region. Magnification, ×630.

FIGURE 7. TSA-FISH detects expression of the golli mRNAs in thymocytes in thymic tissue sections. A, In situ hybridization histochemistry with a golli-specific cRNA probe (green fluorescence) showing pervasive expression of golli mRNAs in thymocytes throughout the cortex and subcapsular region (arrows) of the P6 thymus. B, Nomarski image of tissue section shown in C, in which the TSA-FISH procedure was conducted without the cRNA probe. Magnification, ×630.
negative selection was attributed to a lower and unstable binding capacity for a certain epitope to a particular MHC II molecule (27). Previous work on thymic selection indicates that for any self Ag, expression and presentation by different thymic cells will significantly affect the shaping of the autoreactive T cell repertoire (28). In this regard, our findings of the lack of golli immunoreactivity in stromal cells and the presence of this immunoreactivity in thymocytes are significant. MHC II+ thymic stromal cells (macrophages, dendritic cells, and cortical epithelial cells) are responsible for the negative selection of CD4+ T cells (28–31), whereas MHC I+ thymic epithelial cells, including thymocytes themselves, are responsible for the negative selection of CD8+ T cells (32). Given the lack of significant golli expression in thymic MHC II+ stromal cells, it might be expected that CD4+ autoreactive T cells specific for MBP gene products are generated as a consequence of inefficient negative selection for the CD8+ T cells. As negative selection of CD8+ T cells depends upon the differentiation state of the thymocyte. DP cells are relatively inactive and are programmed to die if they do not undergo positive selection. The majority of DP cells (95%) undergo apoptotic cell death, and only about 5% are rescued from apoptosis by positive selection. These cells differentiate further by selectively down-regulating one of the two coreceptors, CD4 or CD8, and migrate to the medulla, where SP cells are ready to emigrate to the periphery as mature T lymphocytes (21, 22). There are many transitional cells between the DN and DP stages, and the DP and SP stages; CD4lowCD8+ cells lie between the DN and DP stages and CD4+CD8low cells lie between the CD4+CD8− and fully CD4+ or CD8+ SP stages (35–37).

Higher levels of golli were found in DN cells and some transitional DP cells (i.e., CD4−CD8+ and CD4+CD8− cells), but were 2-fold less in the DP cells. Interestingly, when thymocytes survived thymic selection and developed into SP cells, golli protein expression increased again. This pattern of golli expression that golli products may play a role in events that occur during the transition of thymocytes from DN to DP, and from DP to SP stages of development. The subcellular localization of golli in both cytoplasm and nuclei of thymocytes and other unpublished work from our laboratory suggest that golli products may be associated with a signal transduction pathway in T cells and neural cells.

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golli expression changes during thymocyte development

Although recent RT-PCR analysis has suggested the presence of RNA products of the MBP gene in purified rat thymocytes, there was no evidence presented for the expression of the gene at the protein level (33, 34). Our findings indicate that the golli products of the MBP gene are expressed in thymocytes at both the mRNA and protein levels, and that the levels of golli expression change depending upon the differentiation state of the thymocyte.

Thymocyte development can be followed using the CD4 and CD8 T cell surface markers through their least mature CD4−CD8− (DN) stage to their most mature, CD4+ or CD8+ (SP) stage. Associated with this maturation is a change in the localization of these cells within the thymus. For example, DN cells are located in high concentrations in the proliferative, subcapsular zone of the thymus, where they appear to be a precursor population with the capacity of dividing. After proliferation, DN cells differentiate into DP cells and move deeper into the cortex, where they are subjected to thymic selection. DP cells are relatively inactive and are pre-programmed to die if they do not undergo positive selection. The majority of DP cells (95%) undergo apoptotic cell death, and only about 5% are rescued from apoptosis by positive selection. These cells differentiate further by selectively down-regulating one of the two coreceptors, CD4 or CD8, and migrate to the medulla, where SP cells are ready to emigrate to the periphery as mature T lymphocytes (21, 22). There are many transitional cells between the DN and DP stages, and the DP and SP stages; CD4lowCD8+ cells lie between the DN and DP stages and CD4+CD8low cells lie between the CD4+CD8− and fully CD4+ or CD8+ SP stages (35–37).

Higher levels of golli were found in DN cells and some transitional DP cells (i.e., CD4−CD8+ and CD4+CD8− cells), but were 2-fold less in the DP cells. Interestingly, when thymocytes survived thymic selection and developed into SP cells, golli protein expression increased again. This pattern of golli expression that golli products may play a role in events that occur during the transition of thymocytes from DN to DP, and from DP to SP stages of development. The subcellular localization of golli in both cytoplasm and nuclei of thymocytes and other unpublished work from our laboratory suggest that golli products may be associated with a signal transduction pathway in T cells and neural cells.

**FIGURE 8.** Expression of golli mRNAs in thymic tissues and cells de-
tected by RPA. A, golli mRNAs were detected in postnatal brain and liver at P6 and P24. Interestingly, expression of golli mRNAs in the thymus was as high as that in the brain. P6 liver exhibited slight expression of golli mRNAs, which disappeared by P24. B, Levels of golli mRNAs in isolated thymocytes (P6) were high and almost equal to the signal obtained from the equivalent whole thymus (P6, thymocytes + stromal components). The significant reduction in golli mRNAs in the whole thymus after DEX injection provides further evidence of localization in thymocytes, which are destroyed by DEX treatment.


