# THYMIC HORMONE-CONTAINING CELLS

## Characterization and Localization of Serum Thymic Factor in Young Mouse Thymus Studied by Monoclonal Antibodies

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The serum thymic factor (FTS) has been chemically characterized by its capacity to induce T cell markers on bone marrow cells (1). It is a nonapeptide whose amino acid sequence has been determined (2). Recently, we demonstrated (3) that FTS binds the metal zinc that appears to be essential for the biological activity of the hormone. Lastly, data from our laboratory and other laboratories have shown that FTS binds to high-affinity receptors (4) and induces a whole set of T cell markers and functions (1).

The thymic origin of FTS is indicated by its presence in thymic extracts (5). Moreover, using immunofluorescence, the presence of FTS within thymic epithelial reticular cells (ERC) has been recently reported in mouse and man (6–7), using different anti-FTS antisera. These data have been confirmed by immunoelectron microscopy (8). Nevertheless, newly published evidence of an FTS or FTS-like immunoreactivity in several other epithelial tissues (9) has raised some doubts as to the thymic specificity of FTS origin.

In the present study, we describe the characterization and distribution of FTS-containing cells in mouse thymus, as revealed by anti-FTS monoclonal antibodies. The thymic specificity of FTS-containing cells is demonstrated by the negative results obtained with several other epithelial tissues.

## Materials and Methods

*Preparation of Anti-FTS Monoclonal Antibodies.* Four different monoclonal antibodies ($IgG_1$, $IgG_{2a}$, and IgM) were produced (10). The concentration of monoclonal immunoglobulin (Ig) present in the ascitic fluid varied between 6 and 15 mg/ml.

*Immunofluorescence Technique.* 20 mice of the C57BL/6 strain, aged 4–6 wk, were used. The thymus was excised and frozen in liquid nitrogen. Unfixed cryostatic sections (2 μm thick) were processed as described elsewhere (7, 10). As controls, thymus sections were incubated with (*a*) GAM-FITC conjugate only; (*b*) anti-FTS ascites previously absorbed with synthetic FTS ($10^{-3}$ M) overnight at 4°C and 30 min at 37°C; and (*c*) two unrelated antibody-containing ascites.

To ascertain the thymic specificity of the FTS+ cells, sections of spleen, skin, esophagus, stomach, gut, liver, trachea, kidney, urinary bladder, and salivary glands, obtained from both normal and nude mice, were submitted to the four anti-FTS ascites. To determine whether the same cell was able to fix two different anti-FTS monoclonal antibodies, we performed double-

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labeling experiments, using GAM-FITC and GAM-$IgG_1$/TRITC as conjugates. The possibility that one anti-FTS ascites could recognize some but not all FTS+ cells was examined by counting the number of fluorescent cells both after incubation of sections with a single ascites and with a pool of the different anti-FTS ascites.

*Characterization of FTS-immunoreactive Cells*

The epithelial nature of FTS-immunoreactive cells was approached by performing a double labeling of thymic sections and of cultured thymic reticular cells with an anti-FTS ascites and an anti-keratin xenoantiserum and by studying thymic phagocytes as controls.

THYMIC RETICULAR CELL CULTURES. Thymic reticular cells were cultured as described elsewhere (11). In brief, C57BL/6 thymus fragments were cultivated for 12 d in multiwell tissue culture plates (Linbro Chemical Co., Hamden, CT) containing plastic coverslips.

ISOLATION OF THYMIC MACROPHAGES. Thymic macrophages were concentrated on a Ficoll gradient as described previously (12). Cells of the lighter layers were distributed onto the 15-mm plastic coverslips in the wells of Linbro plates. 4 h later, coverslips were recovered and the adherent cells were studied by immunofluorescence.

## Results

*Demonstration of FTS-immunoreactive Cells in the Thymus*

FROZEN SECTIONS. The four anti-FTS-containing ascites succeeded in demonstrating the presence of fluorescent cells within the thymic parenchyma (Fig. 1). The same patterns of brightness as well as the number of positive cells were observed with dilutions of ascites ranging from 1/1 to 1/100,000. All control experiments, including the absorption of the anti-FTS ascites with FTS, were negative. All other organs, either from normal or nude mice, were also negative. Double labeling, using different

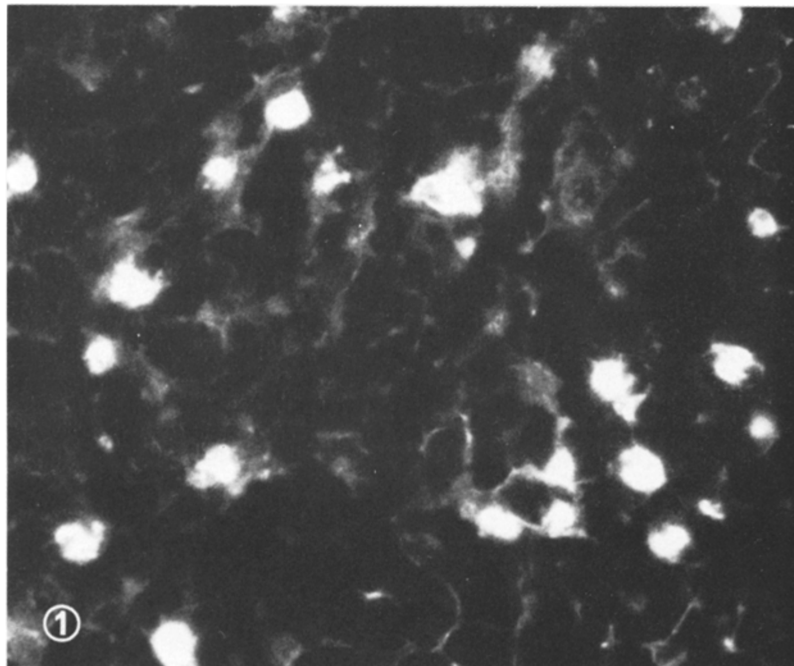


FIG. 1. 5-wk-old C57BL/6 mouse thymus. The frozen section was incubated with an anti-FTS monoclonal antibody and then with GAM/FITC. Several FTS+ cells can be observed. × 500.

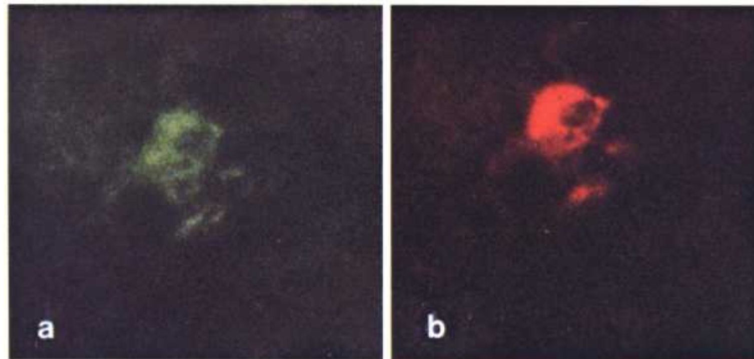


FIG. 2. Double-labeling immunofluorescence on a C57BL/6 mouse thymus section. An anti-FTS monoclonal antibody of the $IgG_{2a}$ subclass was visualized by a GAM/FITC (a), whereas an $IgG_1$ anti-FTS monoclonal antibody was visualized by a GAM ($IgG_1$)/TRITC (b). The same cell is marked by both anti-FTS antibodies. × 750.

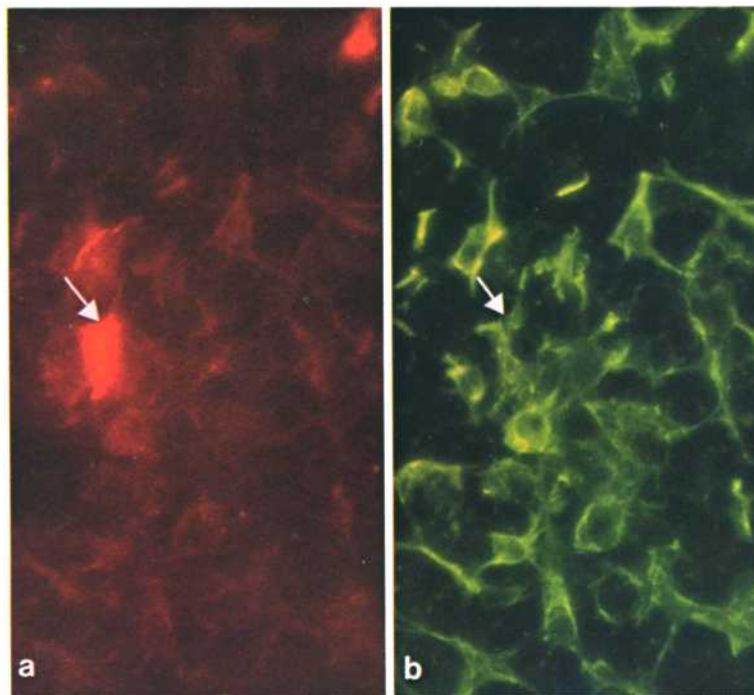


FIG. 3. Double-labeling immunofluorescence on a section from a C57BL/6 mouse thymus. An $IgG_1$ anti-FTS monoclonal antibody was visualized by a GAM ($IgG_1$)/TRITC (a), and a purified anti-keratin antiserum was visualized by a GAR/FITC (b). In the medullary region of this thymic lobule, among the many epithelial cells labeled by the anti-keratin antiserum, one cell (arrows in a and b) contains FTS. × 500.

anti-FTS ascites, showed that the four monoclonal antibodies recognized the same cells (Fig. 2). Furthermore, approximately the same number of FTS-containing cells was found after incubating the sections with either one ascites or a pool of the four ascites.

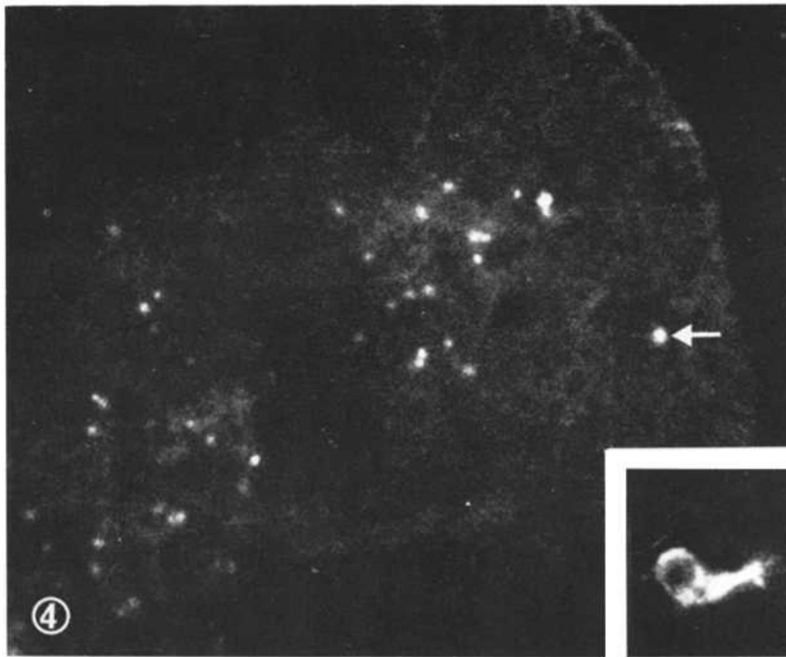

FIG. 4. Thymus section from a C57BL/6 mouse. The anti-FTS monoclonal antibody was visualized by a GAM-FITC. This low magnification micrograph shows the topographic distribution of FTS-containing cells within the thymic lobule. The greater majority of FTS+ cells are concentrated in the medullary region, although one cell (arrow) can be seen in the cortex. × 150. Inset, an FTS+ cell showing a cytoplasmic process and a cell body in which the nucleus appears as a negative image. × 500.

*Thymic Reticular Cultures.* FTS+ cells could also be demonstrated in cultures of thymic reticulum. Immunofluorescence was seen in cytoplasmic granules of the cells. As observed for frozen sections, all controls were negative.

*Characterization of the Epithelial Nature of the FTS-immunoreactive Cells.* Double-labeling experiments using an anti-FTS ascites and an anti-keratin antiserum revealed that FTS-containing cells fixed the anti-keratin antibody, but their number was largely inferior to the total number of keratin-positive cells (Fig. 3). Similar results were observed on cultured thymic reticular cells. The anti-FTS antibody did not bind to isolated thymic macrophages used as controls.

*Distribution of FTS-immunoreactive Cells in the Thymus.* Although they were observed in both the cortex and medulla of thymic lobules, FTS+ cells were found to be much more frequent in the medullary region, where ~1% of the epithelial cells were labeled (Fig. 4). Comparative evaluation indicated that the number of FTS+ cells was about ten times higher in the medulla than in the cortex. The shape and size of FTS+ cells varied, but slender fluorescent cytoplasmic processes were the most frequently observed characteristic. In some FTS+ cells, the cytoplasm showed fluorescent granules present in the cell body and in the cytoplasmic processes. Rare Hassall's corpuscles could be seen in the mouse thymus; some of them showed FTS-immunoreactivity in their periphery.

## Discussion

This paper provides the first characterization and topographic localization of

thymic hormone-containing cells in the thymus using monoclonal antibodies. The use of FTS-reactive monoclonal antibodies and adequate controls assessed the specificity of the immuno-histologic technique. These results confirm and extend those recently reported using xenogeneic anti-FTS rabbit antisera (6–7). The fact that mouse antibodies reacted well with mouse thymic epithelial cells suggests that the reactivity of FTS with the monoclonal antibodies is essentially of the autoimmune type. It must be noted, however, that the same antibodies have been recently shown to react with human thymus (unpublished data). The FTS-containing cells detected in this study should be considered as thymic epithelial cells because they are also marked by anti-keratin antibodies that label only epithelial cells (13). Such epithelial nature was demonstrated in tissue sections as well as in cultures of thymic epithelial cells. The possible role of purified thymic phagocytes was excluded, as they remained unmarked by the anti-FTS monoclonal antibody.

Furthermore, FTS-positive cells were consistently Ia negative (data not shown). This observation, together with the data mentioned above, leads to the hypothesis that FTS-containing cells belong to a subpopulation of thymic epithelial cells included in the minor subpopulation of Ia-negative epithelial cells.

The serum thymic factor is not the only lymphocyte-differentiating peptide produced by the thymic epithelium. One may assume that distinct cells are specialized in the production of each peptide. Such possibility has recently been suggested by Hirokawa et al. (14), who showed that human thymic epithelial cells containing thymosin-$\alpha$1 and thymosin-$\beta$3 are distinct cells and are located in different areas of the thymic lobules. Another possibility (not excluding the preceeding one) is that FTS-producing cells store various amounts of FTS and that at a given time only a minority of these cells are revealed by immunofluorescence.

Our study provides some insight into the distribution of FTS-containing cells in the thymic parenchyma. FTS-positive cells are located in the cortical and medullary regions of the thymus, confirming the results previously found with polyclonal xenogeneic anti-FTS sera (7). On the other hand, the heterogeneous distribution of FTS+ cells within a given thymic lobule, with a strong predominance of these cells in the medulla, has not been described before. This distribution is in keeping with the well-known concentration of epithelial reticular cells in the medulla.

Last, this work brings further direct arguments in favor of the exclusive thymic origin of FTS. We did not find any positive binding of the anti-FTS monoclonals in any other epithelial tissues. This finding fits with those of Monier (6) and Jambon (7) but is at variance with the recent report by Kato et al. (9), who described the presence of FTS-immunoreactive cells in several epithelial tissues. It is possible that the anti-FTS sera produced by Kato et al. recognize an antigenic determinant that, although present on the FTS molecule, is not recognized by our monoclonal antibodies or other xenogeneic antisera. Such determinant could also occur in some FTS-related substances common to several epithelial tissues.

In any case, the fact that, when using our FTS-specific monoclonal antibodies, we detect FTS+ cells only in the thymus, and that FTS disappears from serum after thymectomy, demonstrates the exclusive thymic production of the FTS molecule.

## Summary

The characterization and distribution of cells containing the serum thymic factor (FTS) in the thymus of young mice was studied by immunofluorescence using

monoclonal anti-FTS antibodies. FTS+ cells were distributed throughout the thymic parenchyma but were more frequent in the medullary region than in the cortex.

FTS-containing cells presented a stellate or globular aspect, and some of them exhibited fluorescent cytoplasmic granules. The epithelial nature of FTS+ cells was confirmed by double-labeling experiments using an anti-keratin antiserum (as an epithelial cell marker). Nevertheless, only a minority of keratin-positive epithelial reticular cells contained FTS.

All controls, including the incubation of sections from nonthymic tissues with the anti-FTS antibodies, were negative. Taken together, these results confirm the exclusive localization of FTS-containing cells within the mouse thymus.

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