Macrophage infiltration into thyroid follicles: an immunohistochemical study using donated elderly cadavers

By

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Summary: To describe and discuss the morphology of the aged thyroid gland, with particular reference to the contribution of macrophages. With the aid of immunohistochemistry, we examined 1) macrophage accumulation, 2) infiltration of lymphocytes, and 3) the size and density of follicles in the unilateral lobe of the thyroid gland obtained from elderly donated cadavers (mean age, 84 years) without macroscopic malignancy. Each almost entire unilateral lobe of the thyroid showed 2554–9910 follicles per section, and each of the follicles ranged in area from 0.014–0.072 mm². We often found evidence suggesting absorption and fusion of follicles to provide a larger colloidal lumen, containing small follicles and/or epithelial fragments. In addition to dendritic perifollicular macrophages, large and round macrophages often formed clusters in the colloid. Colloidal lumina with weak macrophage immunoreactivity were intermingled with those showing strong reactivity. Notably, a greater number of macrophage foci in the colloid was usually associated with a lower density of perifollicular macrophages. Likewise, perifollicular macrophages were not always associated with lymphocyte infiltration. In the elderly, the initial appearance of colloidal macrophages does not appear to be associated with perifollicular infiltration of mononuclear cells. Macrophage invasion into a follicle might depend on the functional state of each follicle. After destruction of a follicle, a macrophage cluster appears to remain in the perifollicular tissue, and perhaps lymphocyte infiltration occurs secondarily. This course is likely to represent the process of degeneration of the thyroid gland structure with age.

Introduction

Infiltration of macrophages and/or lymphocytes into thyroid follicles is known to occur in thyroiditis, and lymphocytes often infiltrate the perifollicular connective tissue rather than the follicle itself. Apoptosis is necessary for maintenance of thyroid homeostasis, and becomes evident in inflammation. However, destruction of follicles due to accumulation of macrophages in the follicular lumen has not yet been reported. In our recent study of synovial macrophages in laryngeal joints, we incidentally noted marked accumulation of macrophages in the colloidal lumen. Consequently, the aim of the present study was to describe macrophage morphology in the aged thyroid gland, especially the follicle, with the aid of immunohistochemistry.

Materials and Methods

This study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). Twenty donated cadavers (11 males and 9 females; age at death 74–91 years; mean 84 years) were examined. From each of the cadavers, we obtained the largest sagittal section of the unilateral lobe of the thyroid. All cadavers had been donated to Tokyo Dental College for research and education on human anatomy, and had been fixed by arterial perfusion of 10% v/v formalin solution and stored in 50% v/v ethanol solution for more than 3 months. The use of the cadavers for research was approved by the college ethics committee. The cause of death was ischemic disease of the heart or brain, and during dissection to obtain the specimens, we found no macroscopic pathology in the head, neck.
or thorax. We prepared paraffin-embedded sections of the thyroid lobe. Most of the sections were stained with hematoxylin and eosin (HE), and some of them were used for immunohistochemistry.

The primary antibodies used were 1) mouse monoclonal anti-human CD68 KP1(1:100, Dako M0814, Glostrup, Denmark); 2) mouse monoclonal anti-human CD3 (1:100; Nichirei 413591, Tokyo, Japan); 3) mouse monoclonal anti-human CD8 (1:100; Dako N1592, Glostrup, Denmark); 4) mouse monoclonal anti-human CD79a (1:40; Dako M7050, Glostrup, Denmark); and 5) mouse monoclonal anti-human CD68 KP1(1:100, Dako M0814, Glostrup, Denmark). On the basis of our recent studies using donated cadavers\(^4,6\), the antibody No. 1 was chosen for macrophages, Nos. 2 and 3 for T lymphocytes, and No. 4 for B lymphocytes. The secondary antibody (Dako Chem Mate Envison Kit, Dako, Glostrup, Denmark) was labeled with horse radish peroxidase (HRP), and antigen-antibody reactions were detected via the HRP-catalyzed reaction with dianinobenzidine. Counterstaining with hematoxylin was performed on the same samples. A negative control without a first antibody was set up for each of the specimens. For all sections, observations and photography were usually performed with a Nikon Eclipse 80, but photos at ultra-low magnification (less than x1 at the objective lens) were taken using a high-grade flat scanner (Epson GTX970) with translucent illumination.

Using photographs (HE staining) taken with a x4 objective, we counted the numbers of follicles per section manually. We also traced the thyroid manually, and scanned it with the aid of PowerpointR. To calculate the areas of the traced elements, the scanned images were processed using the ImageJ program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/ij/). Perifollicular macrophages were counted manually per visual field using a x20 objective lens.

### Results

Manual counting revealed that the total number of follicles in each section of an almost entire unilateral lobe of the gland ranged from 2554 to 9910 (Table 1). Using this number, the size of one follicle was estimated to range roughly from 0.014 to 0.072 mm\(^2\). For this estimation, we measured the sectional area of the unilateral lobe including not only the follicles but also the perifollicular connective tissue. When specimens with the minimum and maximum areas (0.072 mm\(^2\); 0.014 mm\(^2\)) were eliminated from the calculation, the mean area of a single follicle was 0.039 mm\(^2\). The colloid that had accumulated in these follicles exhibited differences in density and tintorial quality, ranging from acidophilic to basophilic (Fig. 1).

One or multiple goiter-like clusters or nodules of large follicles were sometimes evident (2 males and 3 females; Table 1; Fig. 1B). The size of the nodules ranged from 0.8 to 14.5 mm; the largest nodule was found in a 74-year-old woman. In two specimens with large nodules (specimen Nos. 1 and 12 in Table 1; Fig. 2), the mean estimated area of one follicle was large (0.072 or 0.068 mm\(^2\)). In the nodule, large follicles tended to be irregular in shape, while small follicles were almost always round or oval, since the former appeared to absorb the latter (see the third paragraph below). Goiter-like nodules contained a small amount of perifollicular connective tissue with or

| Specimen (age, gender) | Follicle numbers (1 follicle, mm\(^2\)) | Goiter-like nodule of follicles | Macrophage focus | Lymphocyte focus |
|------------------------|----------------------------------------|--------------------------------|------------------|------------------|
| No.1 (74F)             | 5330 (0.072)                           | 1 (LL)                         | 15 (+)           | 0                |
| No.2 (76M)             | 4593 (0.045)                           | 0                              | 5 (+)            | ND               |
| No.3 (77M)             | 6013 (0.037)                           | 0                              | 14 (+)           | 5                |
| No.4 (78F)             | 7959 (0.041)                           | 0                              | 152 (+)          | 0                |
| No.5 (80M)             | 6026 (0.023)                           | 0                              | 33 (-)           | 3                |
| No.6 (80F)             | 3420 (0.028)                           | 0                              | 15 (+)           | 0                |
| No.7 (81M)             | 7947 (0.034)                           | 0                              | 5 (-)            | 0                |
| No.8 (82F)             | 2554 (0.046)                           | 0                              | 42 (-)           | 2                |
| No.9 (83M)             | 4873 (0.059)                           | 0                              | 30 (+++)         | ND               |
| No.10 (85M)            | 2706 (0.034)                           | 0                              | 256 (-) (Fig.2)  | 13               |
| No.11 (85F)            | 4005 (0.045)                           | 0                              | 6 (-)            | ND               |
| No.12 (87M)            | 5223 (0.068)                           | 4 (L, M)                       | 53 (+++)         | 20 (Fig. 3)      |
| No.13 (87F)            | 7667 (0.035)                           | 6 (M, S; Fig.1)                | 35 (+++)         | 3                |
| No.14 (88M)            | 5265 (0.039)                           | 0                              | 10 (-)           | 0                |
| No.15 (88F)            | 9910 (0.014)                           | 1 (M)                          | 17 (-)           | 0                |
| No.16 (89M)            | 9108 (0.024)                           | 4 (S, M)                       | 24 (+++)         | 4                |
| No.17 (90M)            | 6435 (0.028)                           | 0                              | 8 (-)            | 0                |
| No.18 (90F)            | 5963 (0.045)                           | 0                              | 10 (+)           | ND               |
| No.19 (91M)            | 3544 (0.036)                           | 0                              | 35 (+)           | 6                |
| No.20 (91F)            | 7888 (0.026)                           | 0                              | 5 (+++)          | 10 (Fig. 4)      |
Thyroid gland macrophages

We often (8/20) found evidence suggesting absorption and fusion of follicles to create a larger colloidal lumen: 1) a large colloidal lumen containing a single or multiple small follicles (Fig. 1C); 2) a large follicle in which the follicular cell layer bore a peninsula-like epithelial protrusion into the lumen and/or fragments of the epithelium (Fig. 1D–F). However, such expanding colloidal lumina without vessels.

Fig. 1. Thyroid gland of an 87-year-old woman. Specimen No. 13 in Table 1. HE staining. Panel A shows a lower-magnification view including an almost entire unilateral lobe of the gland. Panels B–F are higher-magnification views of the squares in panel A. A goiter-like cluster or nodule of follicles (panel B) can be seen in the lower part of the section. The insert at the center of the figure (immunohistochemistry for CD68) exhibits an area with the maximum density of CD68-positive macrophages in the goiter-like nodule. Panel C shows a large colloidal lumen containing smaller follicles (star). Panels D–F show fragments of follicular cells (arrows) present in the colloidal lumen. Scale bars: 10 mm in panel A, 1 mm in panels B and C, 0.1 mm in panels D–F.
carried no or few macrophages and lymphocytes. Unexpectedly, morphologic features suggesting reconstruction of follicles were not evident in the large goiter-like nodules but in the usual follicles.

Perifollicular macrophages were small and dendritic, and their density varied between specimens (low in Fig. 2 and high in Figs. 3 and 4). The density was classified into three grades (−, + and ++ in Table 1). In contrast to the perifollicular dendritic macrophages, we found large, round or oval macrophages in a few or many colloidal lumina in all specimens. When a “macrophage focus” was defined as a colloidal lumen containing CD68-positive multiple large macrophages, these were evident in 15 of 20 specimens (Table 1). The number of macrophage foci varied considerably among specimens, ranging from 5 to 256 per section (Table 1; Fig. 2). The large colloidal macrophages were not associated with T-lymphocytes (Fig. 2A and B). The macrophage foci were classified into two morphologic patterns: 1) large macrophages restricted to and/or occupying colloidal lumina encircled by definite follicular cells (Fig. 2A); 2) absence of all or most of the follicular cells, with large remnant macrophages forming an irregularly shaped cluster (Fig. 2E and F). An intermediate stage to the destruction of follicles was rarely seen: the morphology contained both of the destructing follicles and the accumulation of large macrophage (Fig. 2C and D). Similarly, if strong (or weak) immunoreactivity indicated an active (or inactive) phase, follicles that had already been invaded by macrophages were intermingled with those showing new invasion (Fig. 2A). Macrophages in the gland tissue were most evident when follicle destruction was associated with a high density of perifollicular macrophages (Fig. 2C and D). Notably, however, a great number of macrophage foci in the colloid were usually associated with a low density of perifollicular macrophages (e.g., specimens Nos. 4 and 10; Fig. 2A). Conversely, specimens with a moderate number of colloidal macrophage foci tended to have a high density of perifollicular macrophages (Table 1; Figs. 3 and 4). With a few exceptions (Fig. 3B), goiter-like nodules contained no or few macrophages in both the colloidal lumen and perifollicular tissue (Figs. 1B insert and 3F).

The perifollicular tissue sometimes contained irregularly shaped islands of lymphocyte infiltration (Figs. 3D and E, 4D and E). We found such lymphocyte foci in 9 specimens, and both T and B cells were intermingled within the foci (lymphocyte focus in Table 1; Figs. 3D and E and 4D, E, H and I). However, perifollicular macrophages were not always associated with lymphocyte infiltration. We were unable to perform lymphocyte immunohistochemistry on 4 specimens (ND in Table 1). Moreover, because some sections had been lost during our trials to find the most suitable procedure for lymphocyte immunohistochemistry, sections used for macrophage staining were usually located a little distant from those used for lymphocyte staining (Figs. 3 and 4). There was no apparent gender- or age-related difference in morphologic features of the thyroid follicles, macrophage foci or lymphocyte foci.

Discussion

Textbooks of pathology1) state that in palpable thyroiditis, disrupted follicles contain multiple tiny foci or small granulomas centered around foamy macrophages, lymphocytes and plasma cells. Clusters of foamy macrophages usually extend from the follicular epithelium into the lumen, and most of the lymphocytes (or plasma cells) are T cells (being K-positive). In slight contrast to the traditional pathologic picture, our present observations revealed a spectrum of variation in the foamy macrophages, which appeared as large and round, colloidal macrophages. To our knowledge, no textbooks have yet described the process of follicle destruction after macrophages have accumulated in the colloid. Our present observations showed that 1) the initial appearance of colloidal macrophages was not apparently associated with perifollicular infiltration of mononuclear cells; 2) follicles into which macrophages had already invaded were intermingled with those showing new invasion; 3) after destruction of the follicle structure, macrophage clusters remained in the perifollicular tissue, and lymphocyte infiltration appeared to have occurred secondarily. This course was likely a process of degeneration of the thyroid gland follicular structure with aging.

With a few exceptions, goiter-like nodules contained no or few macrophages in both the colloidal lumen and perifollicular tissue. Moreover, no morphologic features suggesting follicle reconstruction were evident in the large goiter-like nodules, but were seen in the usual follicles. Therefore, goiter-like nodules in the aged thyroid appear to be relatively more stable than the smaller, usual follicles. However, this hypothesis might be inconsistent with Patel et al.7) and Schulte et al.8), who have both suggested active turnover of composite follicular cells in female goiter. In spite of their similar morphologies, the goiter-like nodules in elderly individuals were likely to differ from actual pathologic goiter in terms of development or pathogenesis. Indeed, we found no gender-related differences, although the number of specimens examined was admittedly limited. In addition, macrophage invasion into any given follicle was often unaccompanied by such invasion in the adjacent follicle. Therefore, each individual follicle appears to differ from the adjacent one in its response to colloidal macrophages, and preserves its own stage or functional state.

Faggiano et al.9) measured size of follicles in 31 younger volunteers (maximum age 39 years) and found that most follicles were 40–240 μm in diameter. Since a diameter of 200 micron (the radius or half-diameter...
Fig. 2. Large and round macrophages in the colloidal lumen of the thyroid gland in elderly individuals. Panels A, C–F, immunohistochemistry for CD68; Panel B, immunohistochemistry for CD8. Panel A (from an 85-year-old man; Specimen No. 10 in Table 1) displays abundant foci of large, round, colloidal macrophages: some of the foci appear to be old (clear stars), while others are new (black stars). These macrophage foci do not contain CD8-positive T lymphocytes (panel B, a section adjacent to panel A). Lymphocytes provide a cluster (asterisk in panels A and B). Panels C and D (from a 78-year-old woman; Specimen No. 4 in Table 1) show a rare specimen in which destruction of abundant follicles has occurred with macrophage accumulation (arrows) in combination with perifollicular infiltration of mononuclear cells. Clear stars in panels C and D indicate destructing follicles. Panels E and F (from an 87-year-old woman; same specimen shown in Fig. 1) exhibit foci of large and round macrophages: the follicular structures are destroyed. All panels are prepared at the same magnification (scale bar in panel A, 0.1 mm).
Fig. 3. Thyroid gland of an 87-year-old man. Specimen No. 12 in Table 1. Panel A (HE staining; scale bar, 10 mm) shows a lower-magnification view including an almost entire unilateral lobe of the gland. Three goiter-like nodules of follicles (stars) are evident in panel A. Panels B-I (at the same magnification; scale bar in panel B, 0.1 mm) are higher-magnification views of the squares in panel A. Panels B, F, and H, immunohistochemistry for CD68; panels C, G and I, immunohistochemistry for CD8; Panel D, immunohistochemistry for CD3, Panel E, immunohistochemistry for CD79a. Panels B and C (or Panels F and G; Panels H and I) are adjacent sections, while panels C–E are near sections. Lymphocyte infiltration with abundant perifollicular macrophages is evident in one of the three goiter-like nodules (panels B–E). Some of the macrophage foci in the nodule appear to be old (clear stars), while others are new (black stars). However, the other nodules contain a few macrophages and CD8-positive T lymphocytes (panels F and G). A macrophage focus in the colloid (arrowheads in panel H) is associated with no or few CD8-positive T lymphocytes (panel I). The distribution of CD3-positive T lymphocytes overlaps that of CD79a-positive B lymphocytes.
Fig. 4. Thyroid gland of a 91-year-old woman. Specimen No. 20 in Table 1. Panel A (HE staining; scale bar, 10 mm) is a lower-magnification view including an almost entire unilateral lobe of the gland. Panels B–I (at the same magnification; scale bar in panel B, 0.1 mm) are higher-magnification views of the squares in panel A. Panels B and F, immunohistochemistry for CD68; panels C and G, immunohistochemistry for CD8; panels D and H, immunohistochemistry for CD3; Panels E and I, immunohistochemistry for CD79a. Panels B and C (or Panels F and G) are adjacent sections, while panels C–E (or panels G–I) are near sections. Lymphocyte infiltration with moderate numbers of perifollicular macrophages is evident in this specimen (panels B-I). A macrophage focus in the colloid (arrowheads in panel F) is associated with no or few CD8-positive T lymphocytes (panel G). The distribution of CD3-positive T lymphocytes overlaps that of CD79a-positive B lymphocytes.
being 100 micron) corresponds to 0.031 mm$^2$, our estimated range of 0.014–0.072 mm$^2$ is considered to be within the normal range. Thus, in spite of the presence of large, goiter-like nodules, aged thyroid follicles might not increase or decrease in size. Nevertheless, even physiologically, thyroid follicles tend to change in size. Penel et al.\textsuperscript{10} reported a 76% increase of follicular volume after administration of iodide. Such a change in volume has also been demonstrated in follicles subjected to primary culture\textsuperscript{11}. In a study of rat thyroid using transmission electron microscopy, Uchiyama et al.\textsuperscript{12} demonstrated a daily biological rhythm in which 1) degenerating follicular cells and their cell debris appeared at 16:00 and 2) the colloidal lumen showed a peak volume at 20:00 during 24 hours. They considered that this change in colloidal volume was regulated by absorption and release of the apical cell membrane facing the lumen (reviewed by Uchiyama et al.\textsuperscript{12}). Although it was not a cell level but tissue level, in the present study of the aged thyroid, we have obtained morphological evidence suggesting absorption and fusion of follicles to create a larger colloidal lumen.

The present study demonstrated the expanding follicles as well as the destructing follicles. These morphologies appeared to correspond to two different phases of a life cycle such as development and degeneration. However, conversely, the expanding follicles might not connect with the goiter-like nodule but with the destructing follicles. The goiter-like nodule did not show both processes possibly because of a quite different mechanism.

Conflicts of interest

The authors have no financial conflicts of interest to declare.

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