

Lymph node hyalinization in elderly Japanese

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Summary. Lymph node hyalinization has been comprehensively investigated using specimens obtained from elderly Japanese and white Americans. Onion-peel lesions and associated meshwork areas were often found in the medullary sinus of the thoracic node (mediastinal-type hyalinization), while eosinophilic, glassy and spotty lesions were consistently seen in B lymphocyte areas of the pelvic node (pelvic-type hyalinization). The mediastinal-type hyalinization was comprised of thin collagen fibrils (ca 50 nm in diameter), whereas the pelvic-type hyalinization had thick fibrils (ca 150 nm in diameter). This difference seemed to be consistent with a difference in composite collagen fibrils of vascular walls between the thoracic and pelvic regions. The pelvic-type hyalinization was often or sometimes seen in other nodes, such as cervical, axillary, abdominal and inguinal nodes, especially in white Americans. The mediastinal-type hyalinization, usually in combination with a sinus filled with anthracotic macrophages, tended to be observed in Japanese more frequently than in white Americans. Anthracosis seemed to be connected to the pathogenesis of the hyalinization. On the other hand, because the lesion was weakly positive for Factor VIII immunohistochemistry and because lesions were located along thin vessels, the pelvic-type hyalinization seemed to originate from vascular degeneration in the nodal cortex. Due to the high incidence and large proportion in total volume of the node, the hyalinization seems to be one of the major events that diminish the nodal filtration function and ruin the node with aging.

Key words: Lymph nodes, Hyalinization, Cortex, Sinus, Aging

Introduction

With aging, human lymph nodes tend to become ruined by degeneration such as fatty and/or fibrotic change. Lymph node hyalinization, i.e., onion-peel lesions or eosinophilic deposits in the node, seems to be one of the critical aspects of degeneration. Tsakraklides et al. (1975) described that axillary node hyalinizations increased with age. However, no or few descriptions are found in textbooks except for the comment that "mediastinal and pelvic nodes often carry hyalinization" (van der Valk and Meijer, 1997).

Recently, as the sentinel node procedure has become common all over the world, the individual physiological status of the lymph node, especially activity in trapping of tracers, has been evaluated in detail (Faries et al., 2000). An important issue of this individuality seems to be whether skip metastasis is present or absent. This mode of metastasis has been considered to be a result of collateral lymphatic vessels or bypass of the node (reviewed by Murakami et al., 2002). However, classically, von Ludwig (1962) and Sainte-Marie et al. (1982) reported that specific histological architectures such as an intranodal shunt also allow skip metastasis. We hypothesize that, in aged human nodes, advanced hyalinization injures nodal trapping functions of antigens and/or other particles, which results in an intranodal shunt. However, Anastassiades and Pryce (1966) reported that, in the breast cancer autopsy, axillary nodes with hyalinization tended to carry metastasis. Consequently, the aim of this study is to provide a morphometric and comprehensive description about the individual and region-specific status of nodal hyalinization.

Materials and methods

Specimens

Thirty-five cervical, 26 axillary, 154 thoracic, 106 abdominal and 48 pelvic nodes from a total of 32 sites and 369 nodes (Table 1) were obtained from 27 donated cadavers (12 males and 15 females) aged from 72-95

years old at death. Only one node was picked up at each site. During dissections, the efferent vessels of each node were labeled by carbon particles if possible. These cadavers carried no macroscopic tumors and, according to the death certificates, the causes of death did not include any neoplasms. All specimens were fixed with a 10% formalin solution. Most parts of the present specimens overlapped those used for other recent studies, some of which are now in press (Murakami and Taniguchi, 2003; Sato et al., 2003).

In addition, 150 nodal specimens, obtained from 8-12 sites in 15 white American donated cadavers with almost the same age distribution, were used for the control of parts of this study. These American specimens were not processed for complete serial sections but for cross-sections including the largest cut surface of the node. HE staining and immunohistochemistry were performed. The 150 nodes were a gift from the late Professor Sandy C. Marks, Jr. of the Department of Cell Biology, Massachusetts State University Medical School.

Sectioning

A routine procedure was performed to prepare paraffin-embedded specimens and complete serial sections were made at a thickness of 10 μm . We made 2 axes for 3-dimensional reconstruction according to a conventional method: in short, a paraffin block specimen was prepared as a cube or rectangular block with right angles between any adjacent surfaces and, before deparaffinization, the slide glass was labeled with a sharp glass-cutter at 2 corners of each cubic or rectangular section.

Stainings

Most of the specimens were stained with hematoxylin and eosin (HE), while immunohistochemistry was conducted for several of them. The primary antibodies used in the immunohistochemical evaluation were monoclonal anti-human B lymphocytes CD20 (BioGenex, San Ramon, CA), monoclonal anti-human T lymphocytes CD3 (DAKO A/S, Glostrup, Denmark), monoclonal anti-human macrophages CD68 (DAKO A/S, Glostrup, Denmark), anti-human Factor VIII-related antigen (DAKO A/S, Glostrup, Denmark) and anti-human alpha smooth muscle actin (Dako anti-human smooth muscle; DAKO A/S, Glostrup, Denmark). Two clinically obtained palatine tonsils were used for positive control. No counterstaining was performed. In addition, we performed Bodian's silver impregnation for reticular fibers, phosphotungstic acid hematoxylin (PTAH) staining for fibrinoid deposits and Congo red staining for amyloid deposits for some of those specimens.

Morphometry

Using HE-stained sections, a morphometrical study

of the cortex and hyalinization areas was performed for most of mediastinal and pelvic nodes using 4-5 sections per node. Those included the largest section of all serial sections of the node, with the aid of Photoshop (Adobe, CA) and the NIH image program. The latter was downloaded from the web site <http://esb.info.nih.gov/nih-image/>. We regarded the results obtained from 4-5 slices as a representative of the entire node and, in the present study, we roughly estimated the proportion of lesions in the total volume of the node as "all hyalinization areas/all nodal areas in 4-5 slices." To simplify, we will call this parameter the "proportional volume."

TEM study

In all those specimens, fifteen lymph nodes (mediastinal and pelvic nodes) were divided into 2 pieces and one of the 2 was used for the paraffin-embedded histology as stated above. To prepare Epon 812-embedded specimens, six of the 15 nodes (3 mediastinal and 3 pelvic nodes), in which we made sure of the typical hyalinization by light microscopy, were processed with 2% paraformaldehyde 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for refixation and with 1% buffered OsO_4 for postfixation. After preparing ultrathin sections and contrasting with uranyl acetate and lead citrate, TEM observations were performed using a JEOL 100CX.

Results

Mediastinal node hyalinization

Mediastinal node hyalinization was characterized by its eosinophilic, onion-peel appearance comprising of thick fiber bundles under a light microscope (Fig. 1). The composite fibers were thick and irregularly twisted and branched according to HE staining and Bodian's silver impregnation. In the hyalinization, we did not find either PTAH-positive fibrinoid deposits or Congo red-positive amyloid deposits. This lesion did not have a smooth surface but had irregular protrusions or extensions, which communicated mutually with nearby lesions (Fig. 2). Onion-peel lesions usually had anthracotic CD68-positive macrophages and thin blood vessels (Fig. 1C). These vessels often contained cell debris and various blood cells. The minimum diameter of a single lesion was almost 200 μm if measured in a 2-dimensional section. The maximum diameter was more than 2 mm because multiple lesions sometimes fused together and occupied the entire node (Fig. 1D). Irrespective of whether the onion-peel lesion was large or small, it was consistently located in the medullary sinus, although the sinus should not contain vessels (see Discussion).

According to TEM observations, thick fiber bundles in the onion-peel lesion were composed of thin, cylindrical collagen fibrils 40-50 nm in diameter (Fig. 1E). These thin fibrils were arranged regularly without

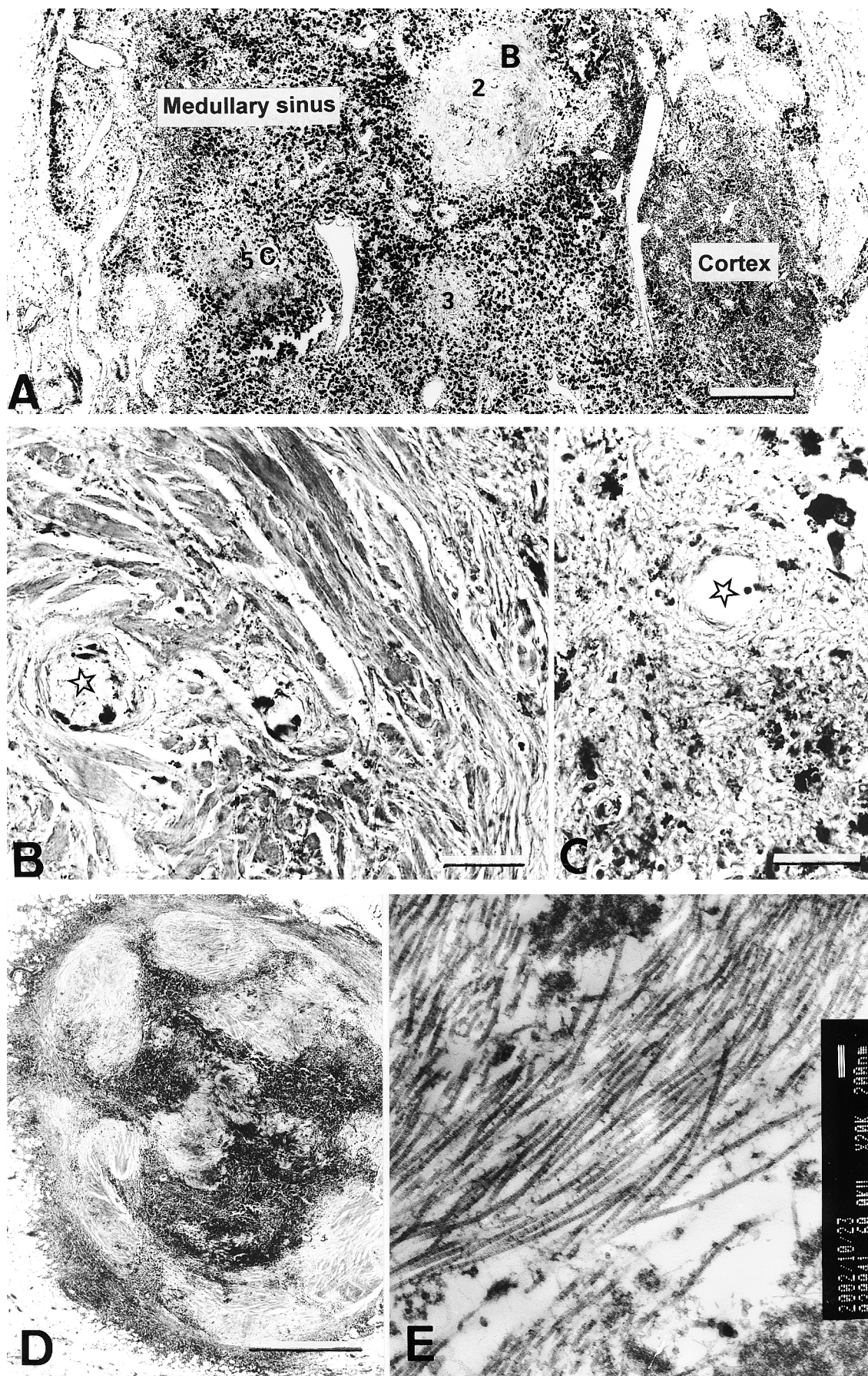


Fig. 1. Histology of the mediastinal node hyalinization, including TEM observation. Paroesophageal node (A-C) and a node ventral to the left pulmonary artery (D and E). H.E. staining (A-D) and TEM observations (E). Labels "B and 2", "C and 5" and "3" for three lesions of hyalinizations in part A correspond to those in Fig. 2. Part B containing blood vessels (open star) and part C showing a thick fiber meshwork are higher magnification views of "B and 2" and "c and 5" in part A. Part E shows thin collagen fibrils comprising of large hyalinizations in part D. Scale bar: A, 300 μm ; B, C, 30 μm ; D, 600 μm ; E, 200 μm .

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any branching. In contrast to roughly arrayed thick fiber bundles seen under the light microscopy, strangely, the TEM observation was quite different (see above). The thin fibrils were accompanied by many deposited substances around and between them. Periodical striations or dark spots were seen in the collagen fibrils at almost 30 nm intervals, but they were unclear.

We often observed an eosinophilic reticular tissue, comprising a thin collagen fiber meshwork (Fig. 1C). It did not contain as many carbon particles and/or anthracotic macrophages as in the usual medullary sinus, and there were even fewer macrophages than in the usual sinus. Thus, the meshwork area looked light or pale pink in the black-colored sinus (Fig. 1A). Notably, in the 3-dimensional view, parts of the onion-peel lesion

consistently attached to, or were even involved in, the meshwork area (Fig. 2). Both the onion-peel lesion and meshwork areas were consistently located in the medullary sinus. The mediastinal node had a very large sinus (usually more than 50% of the nodal volume). Onion-peel lesions tended to be located marginally rather than in the center of the node. In contrast, the meshwork area tended to be seen in the maximum cross section of the node or levels around it. We did not perform TEM observations of the meshwork area.

When small spotty as well as large lesions were also counted, incidences of hyalinization varied significantly in 12 sites in the thoracic region (Table 1). Incidences of more than 80% were found in nodes on the ventral surface of the left pulmonary artery and para-esophageal

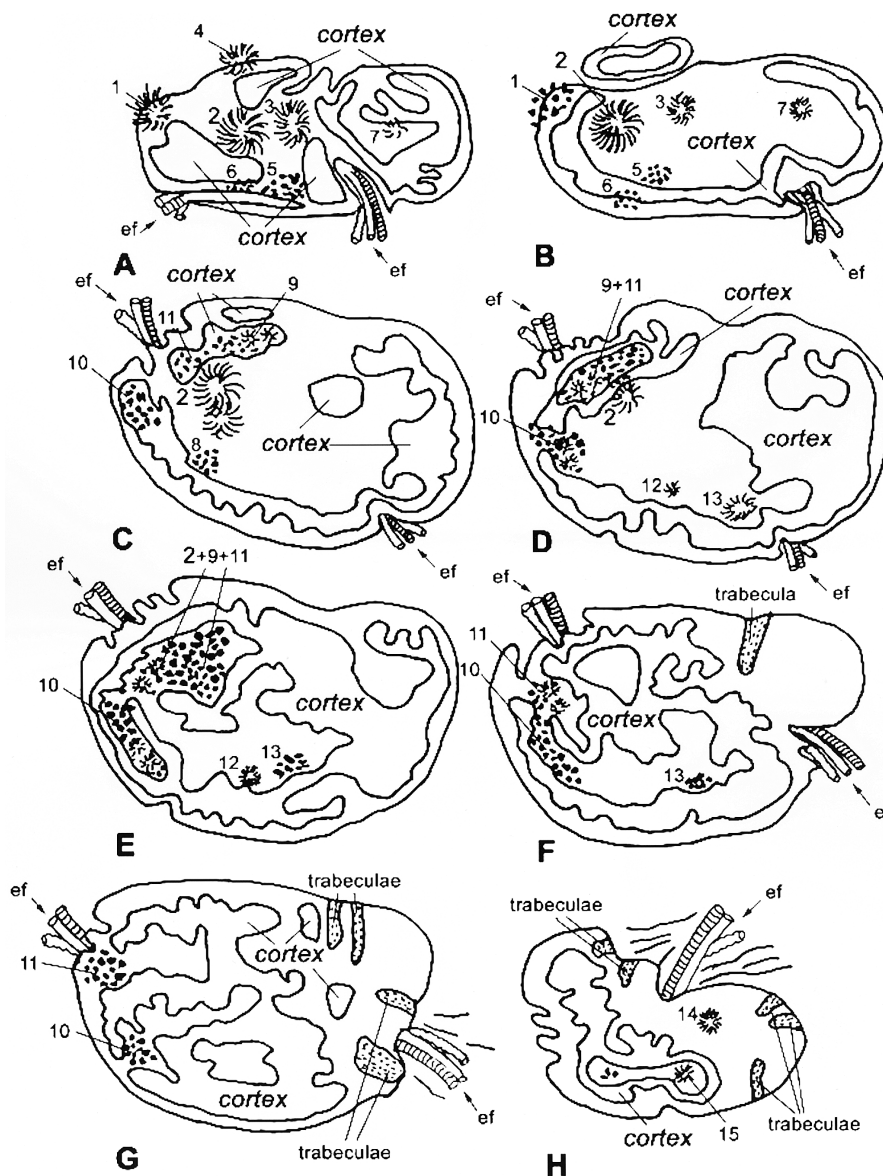


Fig. 2. Diagram showing a 3-dimensional topographical relationship among multiple hyalinization lesions in a single mediastinal node according to observations of serial sections. Para-esophageal node. A sunshine-like label indicates an onion-peel lesion, while a dotted area corresponds to a thick fiber meshwork area (see Fig. 1C). Numerical labels on these lesions correspond between parts of this figure. Parts **A, B, C, D, E, F, G and H** display 0.6, 0.8, 1.0, 1.2, 1.3, 1.5, 1.6 and 2.0 mm levels from the deep or esophageal side of the node, respectively. The maximum diameters of the node were 9.2x4.6 mm and the thickness was 2.3 mm. Thus, part H was located 0.3 mm from the superficial or pleural side of the node. A section shown in part B, including 3 lesions labeled "2, 3 and 5", corresponds to Fig. 1A. Onion-peel lesion No. 2 is the largest of all and it is rugby-ball shaped and 0.9x0.3 mm in diameter. In contrast, Nos. 1, 3-8 and 12-15 range from 0.2-0.5 mm in the maximum diameter. Hyalinization areas are often fused mutually or divided into small pieces. Notably, an onion-peel lesion is almost always adjacent to a meshwork area in the 3-dimensional view. The hili with efferent lymphatic vessels (ef) and feeding arteries differ in location in the node depending on the sectional levels.

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nodes under the level of the tracheal bifurcation. However, these nodes had relatively little hyalinization, i.e., 10-30% of the nodal volume (Fig. 3). Likewise, incidence in the internal mammary node was relatively high (40.0%, Table 1) but the lesion occupied less than 10% of the nodal volume (Fig. 3). A difference between genders was not found.

In multiple nodes at one site of a single cadaver, the size and number of the onion-peel lesions were quite different among these nodes. For instance, one of these nodes had large lesions and associated meshwork areas (see above), while a small lesion was found in the second node and only a meshwork area without an onion-peel lesion in the third node. Thus, in a single site, one or two of the multiple nodes was likely to escape hyalinization, although such an investigation was performed for only 3 sites (3 cadavers).

In mediastinal nodes, we only rarely found the pelvic-type hyalinization (see below) but with a restricted distribution in the node. These cases were

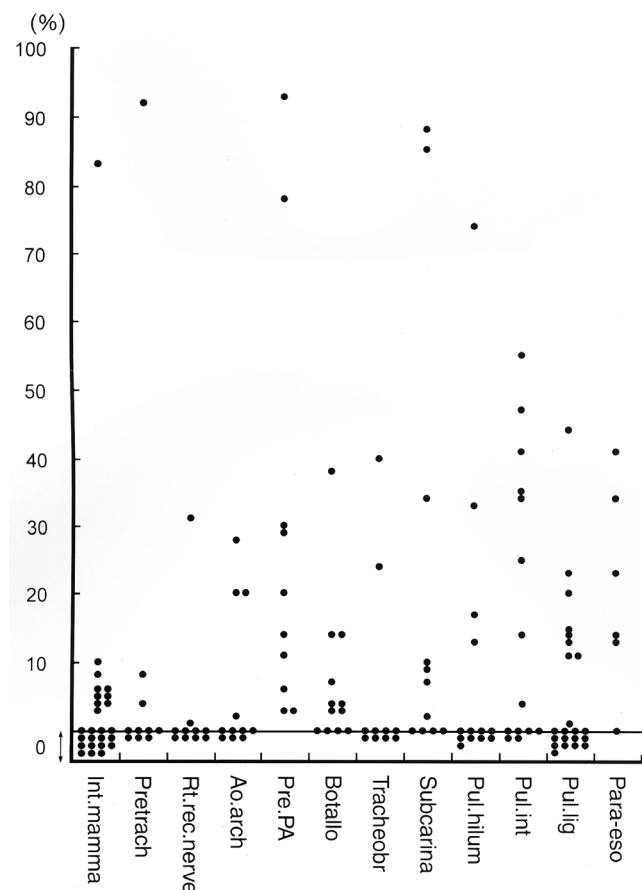


Fig. 3. Proportion of the hyalinization in the total volume of a mediastinal node. Y-axis indicates the proportional volume (proportion of the hyalinization in the total volume of a node). Hyalinization lesions occupied more than 50% of the nodal volume in 8 mediastinal nodes. In contrast, numerous nodes, plotted in "a range" (0 with two-heads-arrows) at the base of the graph, carried no hyalinization.

found in 2 nodes along the left phrenic nerve in the superior mediastinum.

Pelvic node hyalinization

Although pelvic nodes were distributed densely in a relatively small area, we identified the external iliac node (or the obturator node) as a node group along and lateral to the external iliac artery (or adjacent to the obturator nerve). Pelvic nodes almost always (45 of the 48 nodes) carried small, spotty eosinophilic structures in the cortex area including the medullary cord (Fig. 4).

Table 1. Lymph nodes examined and incidences of hyalinization.

SITE OF THE NODE EXAMINED	POPULATED EXAMINED	HYALINIZATION (%)	(%)
Submandibular	15	0	(0)
Subdiaphragic	10	0	(0) [0]
Supraclavicular, upper*	10	1	(10.0) [12.5]
Axillary, central*	13	5	(38.5) [61.5]
Pectoral	13	3	(23.1)
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All cervical and axillary nodes	61	9	(14.8)
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Internal mammary	25	10	(40.0)
Pretracheal	10	3	(30.0)
Right recurrent nerve	10	2	(20.0)
Aortic arch	11	4	(36.4)
Pre-left pulmonary artery	10	10	(100)
Botallo	12	8	(66.7)
Tracheobronchial*	10	2	(20.0) [57.1]
Subcarinal	11	7	(63.6)
Pulmonary hilum*	13	4	(30.8) [64.3]
Intrapulmonary*	14	8	(57.1) [68.8]
Pulmonary ligament*	22	9	(40.9) [62.5]
Para-esophageal, midthoracic	6	5	(83.3)
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All mediastinal nodes	154	72	(46.8)
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Gastric cardia	10	0	(0)
Left gastric artery*	15	3	(20.0) [35.3]
Infrapyloric	11	2	(18.2)
Hepatoduodenal ligament	10	2	(20.0)
Retropancreatic	6	1	(16.7)
Superior mesenteric artery*	9	3	(33.3) [62.5]
Left colic artery	6	2	(33.3)
Ileocolic artery	9	5	(55.6)
Inferior mesenteric artery*	6	0	(0) [90.1]
Middle abdominal para-aortic*	13	12	(92.3) [73.3]
Lower abdominal para-aortic	11	8	(72.7)
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All abdominal nodes	106	63	(59.4)
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Internal iliac artery	13	13	(100)
Obturator nerve*	14	14	(100) [100]
External iliac artery	16	16	(100)
Inguinal*	5	2	(40.0)
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All pelvic nodes	48	45	(93.8)
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All nodes examined	369	189	(51.2)

* Sites examined in white American cadavers [incidence, %].

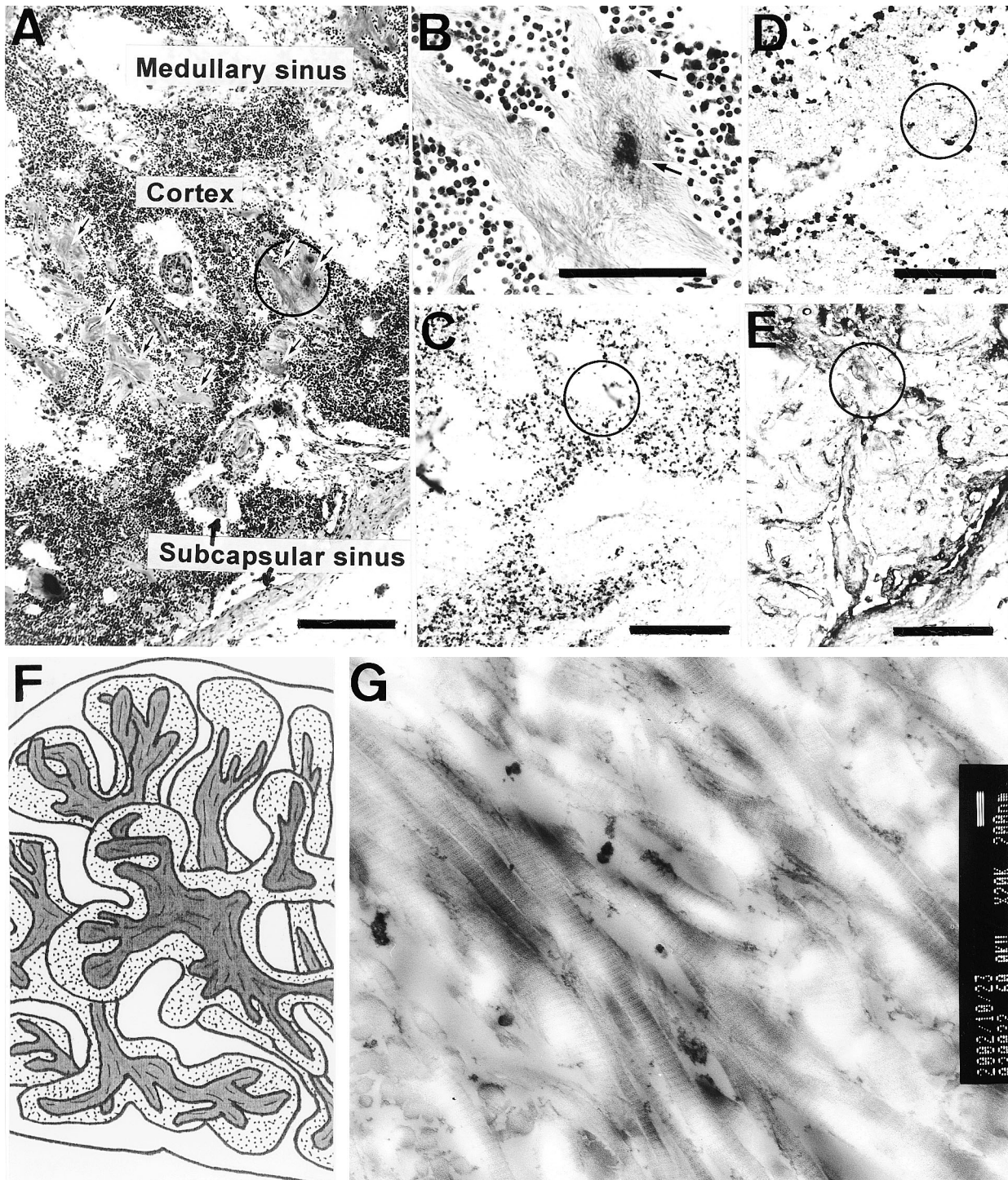


Fig. 4. Histology of the pelvic node hyalinization, including TEM observation. Obturator node. **A-E** display almost the same site. The medullary sinus and cortex are usually intermingled in the pelvic nodes (**A**; HE; bar, 200 μm). **B** (HE; bar, 100 μm) is a higher magnification view of the circled one of numerous hyalinization spots (arrows in part **A**). Note inclusion bodies (arrows in part **B**). **C** (CD3 immunohistochemistry), **D** (CD68 immunohistochemistry) and **E** (Factor VIII immunohistochemistry) exhibit an area including a lesion shown in **B** (circle). These three are the same magnification of part **A** (bar, 200 μm). Note that the T-lymphocyte area faces the subcapsular sinus (part **C**). Hyalinization spots are weakly positive in part **E**. **F** is a schematic 3-dimensional representation of the pelvic node cortex area (dotted area) including a tree-like configuration of hyalinization (dark area). **G** is a TEM observation.

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The spots usually ranged from 20-300 μm in diameter on sections, but in the external iliac node, they tended to be large 2-dimensionally (at maximum, over 1.0 mm), while the obturator node usually had small lesions. We often observed pelvic node hyalinization containing a thin blood vessel or inclusion bodies as its remnant (Fig. 4B). Thus, the hyalinization was weakly positive in Factor VIII-related antigen immunohistochemistry (Fig. 4E). In the cortex and cord, most hyalinization spots were found in B-lymphocyte areas but some of them extended into T-lymphocyte areas (Fig. 4C). Although a low magnification view showed a glassy uniform appearance, under high magnification the hyalinization appeared to contain thin fibers arrayed at random (Fig. 4B). CD68-positive cells were not included in the hyalinization (Fig. 4D). The result for Bodian's silver impregnation was similar to that in HE staining. No deposits were found with either PTAH, Congo red staining or smooth muscle actin immunohistochemistry.

We found multiple foci of the hyalinization at any sections of pelvic nodes, but these lesions were perhaps localized in a restricted area in some sections. When viewed 3-dimensionally, these spotty lesions extended widely and deeply along the long axis of the node. The pelvic node hyalinization sometimes involved a thin vessel in the lesion judging from red blood cells identified in the small lumen. No structural difference was found regarding the intranodal location or on the intrapelvic nodal site. Notably, irrespective of whether the lesions were restricted or distributed diffusely in the nodal sections, all spotty lesions communicated 3-dimensionally to form a tree-like appearance (Fig. 4F). This tree corresponded to a specific, nonlamellar configuration of the cortex areas in the elderly human pelvic node. In short, as also shown in Fig. 4F, multiple tetrapod-like cortices were assembled and connected. According to classical histological views, we found described that, in contrast to a thin superficial cortex, the medullary cord was developed highly to form a complexed interdigitation in the pelvic node (for details, see our other reports). Therefore, in a 2-dimensional section, island-like cortices were distributed in a sea-like sinus area.

TEM observations revealed that fibrous components of the pelvic node hyalinization were composed of thick collagen fibrils 100-150 nm in diameter (Fig. 4G). This thickness was almost 3 times that of the mediastinal-type fibrils. Periodical cross-striations were clearly seen in the collagen fibrils with 40 nm intervals, but the dark band was much wider than in the mediastinal type. The pelvic-type collagen fibrils often branched and were arrayed at random. Thus, these fibrils appeared not to be cylindrical but to have variously enlarged and constricted portions. The matrix material between fibrils was smaller in amount than in the mediastinal-type hyalinization.

The pelvic node hyalinization usually occupied 5-15% of the nodal volume (maximum 20.2%). The proportional of volume in the total cortex volume was

sometimes (12 of all 46 nodes) more than 20% (maximum 40.0%) although the cortex area was much larger than the mediastinal node cortex. Regional differences in the 3 intrapelvic sites, left/right and intergender differences were not evident. According to our additional observations of surgical specimens, 40-50-year-old men also had pelvic node hyalinization.

Hyalinization found in other nodes

Although the size and proportional volume were much smaller than that in the mediastinal and pelvic nodes, nodal hyalinization was often found in sites other than the mediastinal and pelvic nodes (Table 1). Because of the small and restricted lesion (see below), we will call them the "aberrant" hyalinization. Notably, the aberrant hyalinization was limited to the pelvic-type structure, i.e., eosinophilic, glassy uniform structures in the cortex. Thus, in the sinus areas of the other nodes, no lesion with an onion-peel appearance was found. Therefore, the pelvic-type lesion was seen in most sites in the body, while the mediastinal-type was restricted to the thoracic region. However, the "aberrant" pelvic type exhibited a small 3-dimensional extension except for all of the pericolic nodes and some of the abdominal para-aortic nodes. In these exceptional nodes, small spotty lesions were distributed diffusely as seen in pelvic nodes. However, the proportional volume (see Materials and Methods) of the aberrant pelvic-type hyalinization was limited to 1.5-3.0% of the total volume of the node, which corresponded to the lowest group of the actual pelvic type. In the abdominal nodes, strangely, no or little hyalinization was found in nodes at the gastric cardia and along the inferior mesenteric artery (see also next subsection).

Although we had already stated that the aberrant hyalinization showed the pelvic-type structure, an onion-peel-like structure was found in 2 supraclavicular nodes and 1 superior mesenteric node found in three different cadavers. They were weakly eosinophilic and enclosed by a thin capsule. One of the three contained abundant cellular components such as neutrophilic leukocytes. Thus, these were not the usual hyalinization but seemed to be a result of local infection and/or inflammation.

Specimens obtained from elderly white Americans

To compare observations between Japanese and American specimens, we used limited data obtained from the maximum sectional area of the Japanese specimens (see Materials and Methods). Because many fewer sites were examined in the white Americans than in the Japanese, i.e., 8-12 sites per cadaver (at maximum: the subdiaphragmatic, upper supraclavicular, axillary (central), left tracheobronchial, pulmonary hilum and intrapulmonary, pulmonary ligament, left gastric artery, superior mesenteric artery, inferior mesenteric artery, middle abdominal para-aortic, obturator nerve and inguinal nodes), data of Japanese

nodes were also limited to the corresponding sites in this subsection.

The obturator nodes, the only site examined in

pelvic nodes of white American cadavers, consistently carried hyalinization. Each of the spotty pelvic-type lesions was generally larger than in the Japanese.

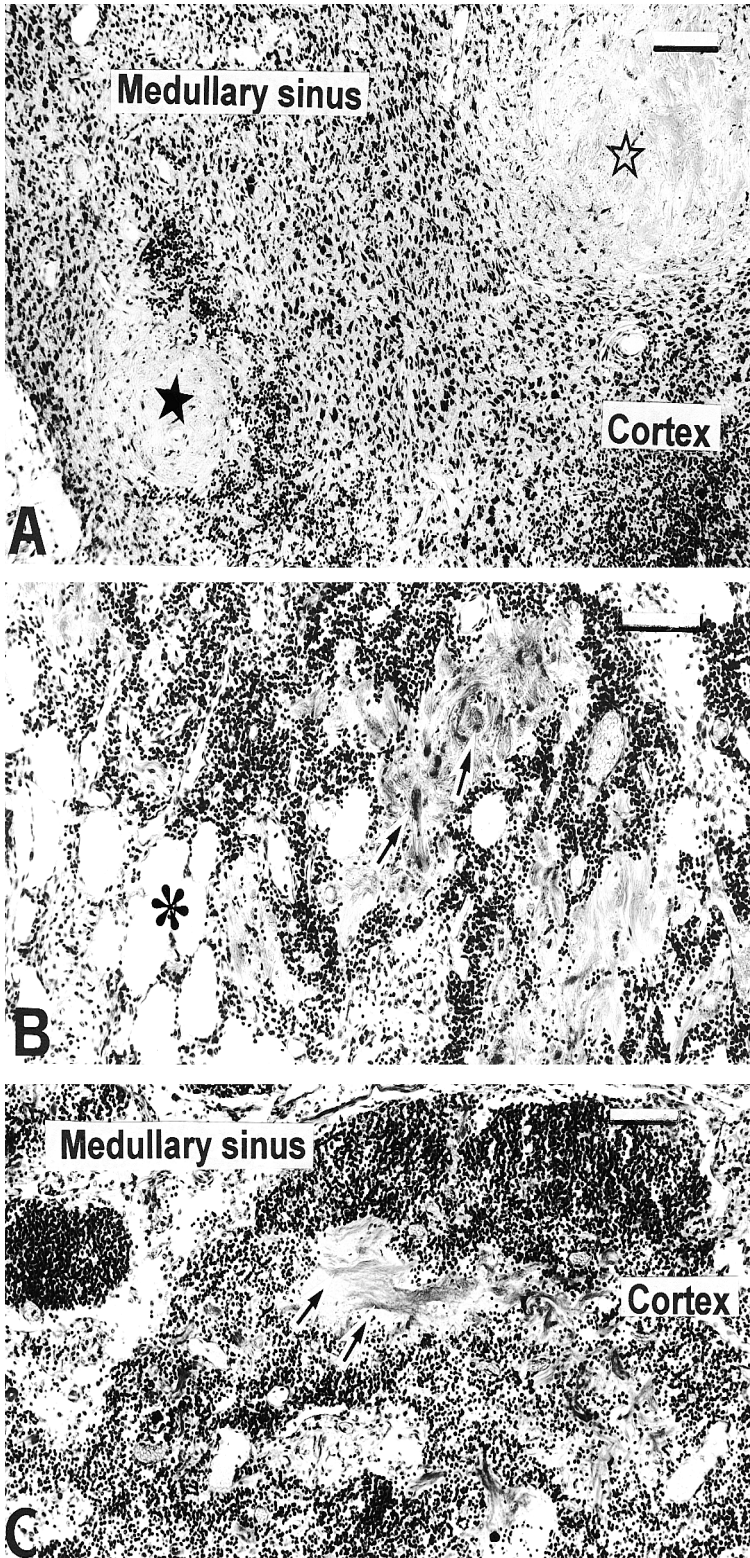


Fig. 5. Lymph node hyalinization in elderly white American specimens. **A** shows a mediastinal-type hyalinization found in the pulmonary ligament node. An onion-peel lesion (open star) and a mesh-like area (black star). In this case, sinus anthracosis is extremely severe in white American specimens. **B** is an inguinal node with fatty change (asterisk) of the cortex. Note inclusion bodies (arrows) in parts A and B. **C** displays a small but rare case found in the axillary node. Fibrous components are clearly seen (arrows). Bars: 100 μ m.

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Remnants of thin vessels and inclusion bodies were seen in the lesions more frequently than in obturator nodes of the Japanese. Notably, rather than in the pelvic node, the American specimens carried pelvic-type lesions in other nodes such as the axillary, inferior mesenteric and inguinal nodes more often than in the Japanese (Fig. 5). This difference was quite evident in the inferior mesenteric nodes (nodes along the proximal portion of the inferior mesenteric artery); 10 out of the 11 nodes had the pelvic-type hyalinization in the American specimens, whereas all 6 nodes (6 cadavers) had no lesion in the Japanese. Instead, the Japanese specimens often showed severe sinus fibrosis in the inferior mesenteric node.

Between these two populations, we found another difference in the incidence and size of the onion-peel lesions in the thoracic nodes. In white Americans, 2 out of the 15 cadavers (13.3%) carried 3 nodes with small onion-peel lesions (3 out of the 60 thoracic nodes or 5.0%). All these lesions in the American specimens were 2-dimensionally small and occupied less than 10% of the sectional area of the node. These three nodes contained many more anthracotic macrophages than usual in the American specimens (see below). The thoracic nodes examined in the American specimens were the left tracheobronchial, pulmonary ligament, pulmonary hilum and intrapulmonary nodes, all of which exhibited relatively low incidences of hyalinizations in the Japanese (Table 1). In the same sites of the Japanese, 4 out of 27 cadavers (14.8%; 12 out of the 59 nodes or 20.3%) had onion-peel lesions of various sizes including large ones occupying more than 20% of the maximum sectional area. Thus, the incidence in population was nearly the same. However, when elderly Japanese had onion-peel lesions, the number per person and the size of one lesion tended to be larger than in elderly white Americans. In white American thoracic nodes, onion-peel lesions were also consistently associated with the meshwork area (Fig. 5 and see above subsection). Conversely, meshwork areas without a concomitant onion-peel lesion were often observed in white American thoracic nodes.

In the thoracic nodes, the numerical density of anthracotic macrophages was usually strikingly smaller than in the Japanese although we did not precisely measure them. Macroscopically, thoracic nodes were often pale grey in white Americans in contrast to black nodes in elderly Japanese, irrespective of smoking habits. In addition, the American specimens often contained rich fatty tissue in the nodal cortex at every site (Fig. 5). The severe fatty tissue invasion, which occupied more than 50% of the nodal volume, was very rare in elderly Japanese (only 5 out of the 369 nodes).

Discussion

Two types of nodal hyalinizations

In the present study, we have revealed regional

differences in the morphology, incidence and proportional volume of the hyalinization. The mediastinal-type hyalinization was characterized by 1) occurrence in the medullary sinus, 2) an onion-peel appearance and 3) its composite thin collagen fibrils. However, the pelvic-type hyalinization exhibited opposite features of 1) cortex-restricted distribution, 2) homogeneous eosinophilic structures and 3) composite thick collagen fibrils. The pelvic-type lesion was somewhat similar to the proteinaceous deposits mimicking Hassall-like bodies seen in the hyaline-vascular type of Castleman's disease (Palestro et al., 1999). However, this specific hyalinization was restricted to the germinal center of the mediastinal lymph node in combination with a change in cellular components of the mantle zone of nodal follicles. Therefore, it seems possible to exclude this pathological change from the present discussion.

Between the mediastinal and pelvic-type hyalinizations, the difference in thickness of collagen fibrils was very evident, i.e., 50 nm in the former and 150 nm in the latter. In general, the diameter of collagen fibrils varies from 10-500 nm, and depends on the location of the tissue and the age and species of the animal (Parry and Craig, 1984). For example, the bovine cornea has collagen fibrils with a regular diameter of about 30 nm, while the diameter of scleral collagen fibrils ranges from 25-230 nm (Komai and Ushiki, 1991; Yamamoto et al., 1997). Although little has been reported about the thickness of vascular collagen fibrils, according to Stehbens and Martin (1993), fibrils ranged from 40-75 nm (mean, 52 nm) in diameter in the aortic adventitia, while they ranged from 40-175 nm (66 nm) in the aortic media. Notably, there was a difference in thickness of vascular collagen fibrils between the pelvic and mediastinal nodes (data not shown in Results). The pelvic node arteries had thick fibrils (150-200 nm) whereas the mediastinal node carried thin fibrils (50-100 nm). The intercostal artery and its branches also had thin fibrils (20-40 nm in diameter, unpublished data). People may consider that, in pelvic nodes and their hyalinization, thin collagen fibrils fuse mutually to make a thick fibril. However, clear cross-striations in the thick fibrils seemed to deny this possibility because simple fusion without reassembly of collagen molecules would make the striation unclear. Reassembly with aging has not been reported in the human body to the best of our knowledge.

Pelvic-type hyalinization and vascular degeneration

Pelvic-type hyalinization seemed to involve a thin vessel in the lesion according to HE and Factor VIII-related antigen immunohistochemistry. In the pelvic node, the 3-dimensional tree-like configuration of the hyalinization also suggested that it occurred along the vascular tree. In pelvic node sections with numerous hyalinization spots, the numerical density of arterioles (or thin arteries with the distinct smooth muscle layer)

was much lower than the high density of postcapillary venules. In the node, the arterial density appeared to be extremely low in the cortex area in which numerous hyalinization spots were concentrated, whereas it was relatively high in other cortex areas with few lesions. Thus, the higher density of pelvic hyalinization seemed to correspond to much less arterial circulation or even arterial obliteration in the area. In general, hyalinization is a common lesion of arterioles and small arteries and increases with age and in conditions such as hypertension and diabetes. The glassy uniform appearance of the arterial hyalinization is considered to be the result of accumulation of a variety of plasma proteins and small amounts of lipids, especially in the vascular media (Gallagher, 1997). However, the hyalinization was negative in smooth muscle actin immunohistochemistry.

For the donated cadavers, we had no information about past history such as hypertension and/or diabetes as a likely background positive for the arterial hyalinization. However, the elderly white American cadavers were much fatter than Japanese and more than half of their causes of death included heart failure, in contrast to pneumonitis as a major cause of death for the Japanese cadavers examined. The present study revealed that the pelvic-type hyalinization spots in white Americans were much larger than in the Japanese. It is likely that pelvic-type hyalinization starts from a vascular degenerative change in the nodal cortex and that the progress depends on blood chemical components such as lipid and cholesterol. We have little information about either human vascular collagen fibrils (see also below) or nodal hyalinization itself in hypertension and/or diabetes, although several studies were conducted using aged experimental animals (Richardson et al., 1995; Wegiel et al., 1995; McGuffee and Little, 1996). The hyalinized vascular change was reported in mucosal prolapse syndrome (Lonsdale, 1993) and anal fibroepithelial polyps (Sakai and Matsukuma, 2002), but these lesions were associated with CD34-positive stromal cells, although the present specimens were not available for immunohistochemistry (data not shown).

Mediastinal-type hyalinization and its associated meshwork structures

Detailed observations of serial sections (Fig. 2) revealed that the mediastinal-type hyalinization was intermingled and surrounded by the eosinophilic meshwork or reticular structure. The meshwork was most likely to be composed of collagen fibrils. Notably, this meshwork area contained fewer anthracotic macrophages than the usual medullary sinus. Although TEM observations were not performed for the meshwork area, the onion-peel lesion contained rich matrices according to TEM observations. Thus, also in the meshwork area, it seemed to be likely that the matrix between meshwork fibers prevented occupation by sinus macrophages. In both Japanese and white American

thoracic nodes, onion-peel lesions were consistently associated with meshwork areas. We observed various "stages" of the mediastinal-type hyalinization in multiple nodes in one site, i.e., large, small and no onion-peel lesions, all of which were associated with the meshwork area adjacent to the lesion. In contrast to Japanese specimens, white American thoracic nodes often carried meshwork areas without a concomitant onion-peel lesion. We hypothesized that the meshwork area corresponded to a stage of matrix deposition in the reticular structure and that it was the beginning of onion-peel lesions. Anthracosis seemed to be connected to the suggestive change from the meshwork to onion-peel lesions. The rate of smokers in the elderly population is more than 60% in Japan (Sapporo City, 2001), which is quite different from that in white Americans.

Fibrosis as well as hyalinization in the vascular media is considered to be a general feature in degenerative changes with aging in vascular walls (Gallagher, 1997). Recently, in abdominal lymph nodes in elderly Japanese people, our group described severe sinus fibrosis starting from the perivascular area, i.e., collagen fiber masses containing abundant fibroblast-like cells occupy the medullary sinus except for the narrow space around the medullary cord (Murakami and Taniguchi, 2003; Sato et al., 2003). The mediastinal-type hyalinization was also restricted to the sinus area. However, it contained few cellular components, even including fibroblasts. Fibrosis in the liver and lung is well known and it seems to be triggered by macrophages or similar cells such as Kupffer's cells (Yang et al., 2003). However, sinus fibrosis in the abdominal node did not seem to be associated with macrophage function because there were no or few macrophages in the lesions at any stages during the fibrotic process (Sato et al., 2003). Likewise, in the small lesion or early stage of hyalinization, we found few CD68-positive cells except for the usual tinged body macrophages and anthracotic macrophages. Thus, the trigger of hyalinization is not likely to exist in macrophages but in vascular walls. Nevertheless, the medullary sinus does not contain any vessels according to classical concept of histology. Therefore, we speculated that, in aged human nodes, the cortex area is reduced in size and that vessels primarily supplying the cortex become exposed to the sinus area.

Nodal function and hyalinization

Does hyalinization disturb the lymph node function? Sato et al. (2003) demonstrated that elderly Japanese people often had "ruined nodes" which seemed not to be suitable for their activity as a local barrier. In these, the nodal superficial cortex was often very thin and fragmented with gaps. This architecture seemed to allow intranodal shunt flow (see Introduction). The paracortex area was difficult to identify. Anthracosis was quite evident in elderly Japanese people, irrespective of smoking, in contrast to white Americans. Fatty tissue

Human lymph node hyalinization

invasion into the nodal cortex and/or medullary sinus was rare in the Japanese but often found in the Americans. The present result suggested that nodal hyalinization is another cause of ruined nodes. The mediastinal-type hyalinization seemed to be problematic for nodal function in elderly Japanese people, while the pelvic-type was evident in the Americans. Furthermore, we hypothesized that these ruined nodes provide pitfalls for the sentinel node procedure because their activity in trapping of tracers is likely to be different from that of metastatic cancer cells. Tracers seem to be trapped by sinus macrophages, whereas dendritic cells and T-cells seem to play an important role in trapping metastatic cancer cells. According to the present results, nodal hyalinization attacks either the cortex or medullary sinus depending on the site of the lymph node.

Classically, Anastassiades and Pryce (1966) demonstrated that, using axillary nodes obtained from the breast cancer autopsy, the hyalinized node tended to carry metastasis. They also described that a negative correlation between sinus histiocytosis and hyalinization in the axillary nodes. However, their results were based on the autopsy. Ruined nodes due to hyalinizations seemed to allow early spread of cancer cells, that resulted in death of patients and autopsy. The hyalinized, less active cortex was not likely to accompany the accumulation of sinus macrophages. Nevertheless, the suggested effect of hyalinizations against the sentinel node concept should occur at the very early stage of metastasis, not in the final status of nodal involvement because of the definition of the concept.

Study limitation

Although we prepared serial sections, the incidence and morphometry were based on 4-5 sections including the maximum sectional area of the node. People may suspect whether those incidences at a specific site corresponded to the "actual incidence" because another portion of the node, outside of the 4-5 sections, might include hyalinization. However, we believe that the present incidence totally represented regional variations because we picked one node at random at every site of a single cadaver. Likewise, the present proportional volume seemed to represent a regional difference although it was not estimated according to the complete 3-dimensional reconstruction. We used specimens obtained from the elderly population. Clinically-obtained specimens suggested that, in 40-50 years of age, both of the mediastinal and pelvic-type hyalinizations had already occurred in some individuals. However, further studies are required to know when and how hyalinization begins. In a future study, data of blood chemicals and body size would be helpful for discussions about the background of the nodal hyalinization.

Finally, we have to state about a limitation for application of immunohistochemistry using specimens obtained from cadavers those were donated for education and research of anatomy. This problem was

likely to be caused by a delayed formalin fixation 24-72 hours after death. Thus, except for the smooth muscle actin and factor VIII, we could not obtain any immunohistochemical evidences on the stromal cell and matrix in and around the hyalinization. Further studies using fresh specimens will reveal which cells, the follicular dendritic cells or other reticular cells, provide the suggested progenitor of the mediastinal-type hyalinization (i.e., the meshwork area) and which type of collagens, rather than type I, is specifically found in and around the pelvic-type hyalinization.

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