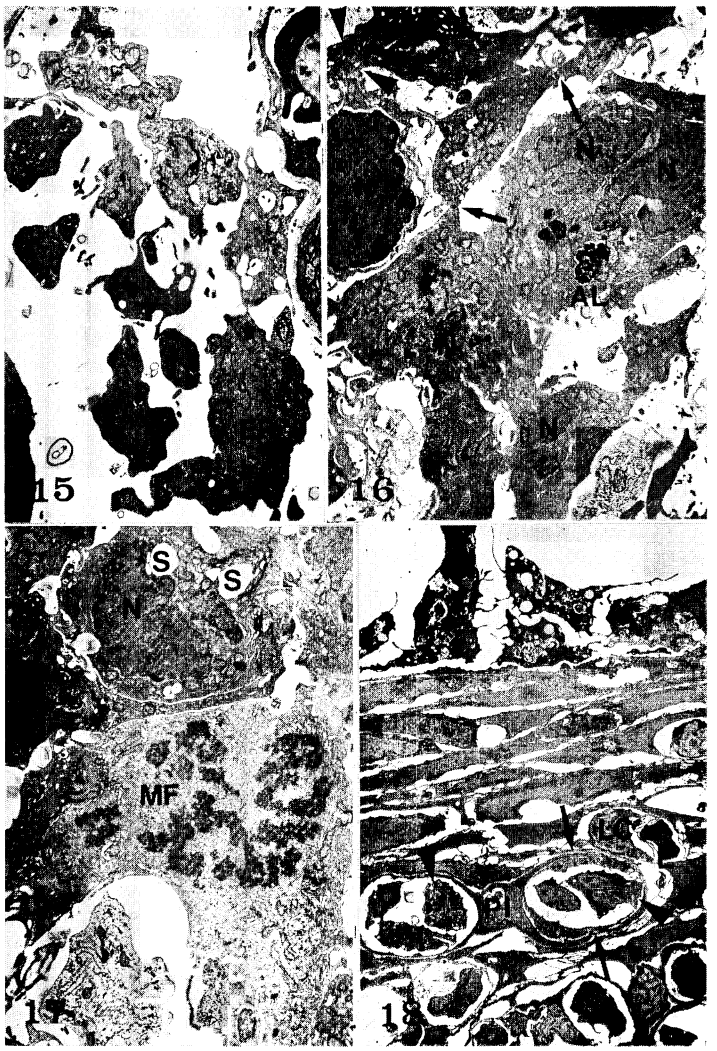

Fig. 15. Fragmented epithelial cell (EP) and its cell debris. ×4,130.

Fig. 16. Transformation and nuclear lobation in epithelial cells. Arrows show non-typical desmosomes. At the left corner an epithelial cell is connected with the cytoplasm at the narrow part (arrowheads). AL, autolysome; N, nucleus. ×4,130.

Fig. 17. Mucous cell shut up in the lesion. MF, mitotic figure; N, nucleus; S, secretory granule. ×4,130.

Fig. 18. The most advanced hyperplastic lesion. This corresponds to region A of fig. 3 (Kudo and Kimura, in press a). The superficial layers are organized by spindle-like epithelial cells. Cells of the outermost layer are exfoliating and contain varying numbers of lysosomes. Arrows show a double layer of flanges in pillar cells. An arrowhead shows unusual cells. E, erythrocyte enclosed in the lesion; LC, lymphocyte-like cell; PI, pillar cell. ×1,260.

were sometimes elongated in shape and could be discriminated from lymphocyte-like cells only by small clusters of smooth endoplasmic reticulum. The epithelial cells were spindle-like or flat in two or three superficial layers, but polygonal or irregular in the interior of the lesion. Their irregular outline was characterized by increased numbers of thin winding processes or thick pseudopodium-like processes which were frequently branched or roughly interdigitated with those of adjacent cells. The phenomenon of cytoplasmic "cutting-away" or "fragmentation"

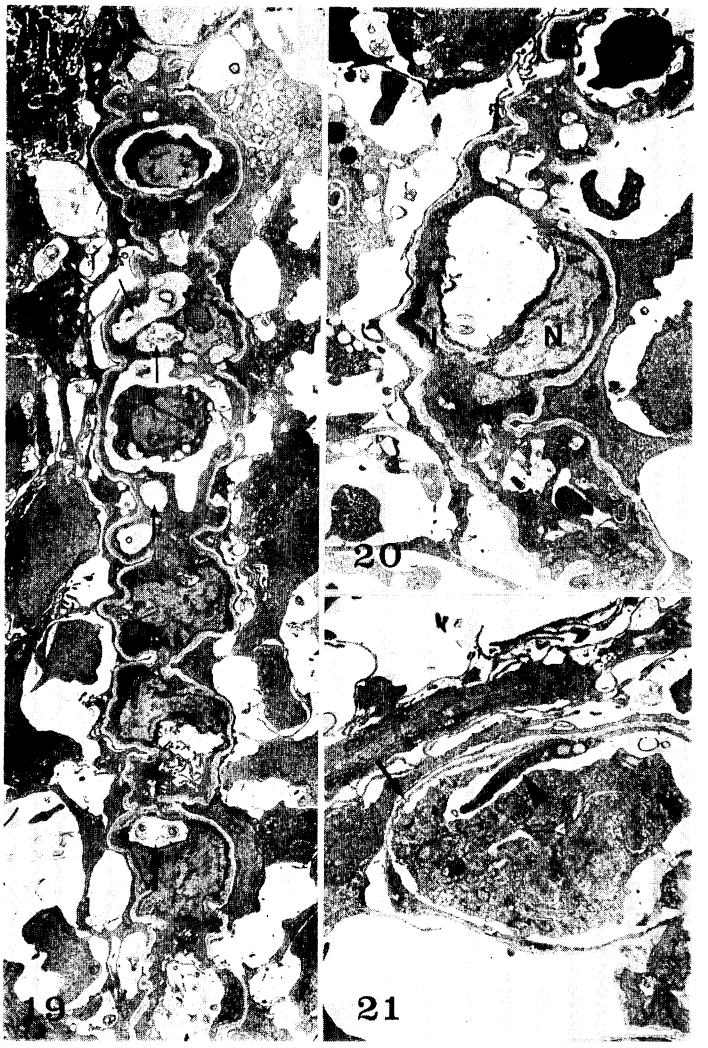

Fig. 19. Strand of pillar cells in bacterial gill disease. The strand is characterized by nuclear pseudomorphs, subdivision of the blood capillary space by thin cytoplasmic projections of the pillar cell (thin arrows), irregularly winding cytoplasmic projections extending from the flanges of the pillar cell, and the space formation with the flanges of a single pillar cell (thick arrow). ×2,660.

Fig. 20. Nuclear lobation (N) (or three nuclei) in a pillar cell. ×3,710.

Fig. 21. Unusual cell in the space surrounded with pillar cell flanges. Part of the unusual cell anchors on the basement lamina (arrow). The other cell flange also extends into the space (arrowhead). ×3,990.

in the cell periphery was also observed. This resulted in the formation of cell debris and simultaneous transfiguration of epithelial cells to roughly oval, lymphocyte-like or spindle-like and variously distorted cells (Fig. 15). It is possible that this transfiguration may contribute to the organization of the superficial layers of spindle-like epithelial cells. Polygonal epithelial cells very often had a di- or tri-lobate nucleus, two or three nuclei on ultrathin sections or a mitotic figure, and autolysomes (Fig. 16), and were connected with non-typical desmosomes only between thick processes of adjacent epithelial cells (Fig. 16). Epithelial cells in the outermost layer had a number of lysosomes or autolysomes and were often exfoliating (Fig. 13). Mucous cells enclosed in the lesions were oval in shape and comparatively smooth in out-

line. They had variable numbers of secretory granules, comparatively developed rough endoplasmic reticulum, Golgi apparatus whose lamellae had very narrow cisternae, a di-lobate nucleus (or sometimes two nuclei on ultrathin sections) and sometimes autolysomes (Fig. 17). Macrophages often contained phagolysosomes of various sizes. The phagolysosome contents varied from a distinguishable to an indistinguishable state of cell organelles. The distinguishable organelles were reminiscent of the debris of either epithelial or chloride cells. The morphology of pillar cells in bacterial gill disease will be described later.

In more advanced lesions, the fusion of two or more gill filaments progressed with morphological changes of organizing cells. Many of the epithelial cells transformed into a spindle-like or fibroblast-like shape so that the superficial layers were organized with several layers of epithelial cells arranged along a longitudinal axis of gill filaments (Fig. 18). The epithelial cells in the outermost layer contained a number of lysosomes or autolysomes and frequently showed exfoliation from the hyperplastic epithelium. Their cytoplasmic matrix appeared more electron dense than that of the spindle-like epithelial cells in the lower layers. The deep interior of the lesion was characterized by the same morphological changes as in the previous stage: nuclear lobation, cytoplasmic "cutting-away" or "fragmentation", the decrease of intercellular contacts, and increased numbers of cytoplasmic processes. This increase might cause the enlargement of intercellular spaces and the decrease of desmosomes between epithelial cells. The desmosomes were indeed not only small in number but also less typical in configuration.

Pillar cells in bacterial gill disease also changed morphologically. Their early changes had already occurred in hypertrophied gill lamellae. In the lesions of remarkable lamella fusion, pillar cells were characterized by nuclear pseudomorphs such as incision or depression (Fig. 19) and lobation (Fig. 20), thickening of the cytoplasmic flanges, the increase of cytoplasmic projections extending from the flanges towards the blood capillary spaces and narrowing of the spaces. The projections were relatively long, irregularly winding and often branching. The space narrowing may be caused by the increase of the projections, subdivision of the space into two or three segments, or formation of the space with the flanges of a single pillar cell (Fig. 19). In more advanced lesions, the flanges of pillar cells were sometimes composed of a double layer (Fig. 18), for closer scrutinization revealed that part of the inner layer was also connected to the basement lamina. Further, in the blood capillary spaces surrounded by the flanges, there frequently appeared cells other than blood cells such as erythrocytes, leukocytes, lymphocytes, monocytes and thrombocytes. The cytoplasm of the unusual cells was similar in texture to the inner layer in a double layer of flanges and they contained two nuclei, sometimes a bi-lobate nucleus or infrequently three on ultrathin sections (Figs. 18, 21). Furthermore, a few of the unusual cells were found to anchor on the basement lamina with part of the cytoplasm or cytoplamic projections and to jut out into the flange-surrounded space (Fig. 21). On the other hand, near the erythrocytes enclosed in the lesion a single cell similar to pillar cells was sometimes found (Fig. 18). In the most serious lesions even the strands of pillar cells were somewhat difficult to find.

Discussion

In healthy, slightly older trout fingerlings than those used in the present examination and in adults, the gill filament (or interlamellar) epithelium consists of several layers of epithelial cells and a considerable number of chloride and mucous cells. The latter were located in the outermost layer of the filament epithelium and were numerous in the transitional or marginal regions between the gill lamella and filament epithelia (Morgan and Tovell, 1973; Kudo, unpublished). Further, the majority of gill lamellae were covered with two layers (proximal and distal) of epithelial cells, whose development as a respiratory functional structure had been completed. In the present fingerlings, however, the epithelium of gill lamellae was still incomplete and a single layer of thick epithelial cells and chloride cells adjacent to pillar cells cover fairly wide surface areas. As recently reported (Kimura and Kudo, 1979), this suggests that gill lamellae in trout fingerlings may have only a narrow area which functions as the respiratory structure. Thus, the appearance of autolysomes

in thick epithelial cells might be closely related to partial degradation or diminution of the cytoplasm. These cells may transform into flattened lamellae epithelial cells as a final morphological step in the spectrum of cell differentiation to respiratory functional cells, in turn contributing to an increase in respiratory functional area. This is thought possible because no signs of exfoliation of the thick epithelial cells from the lamellar epithelium were seen and there were no such cells in adult trout. Morphological variations in the thinning of the thick cells were closely correlated to the decrease of vacuoles (or autolysomes) in the cytoplasm and of membranous elements in the intercellular spaces. These findings contradict the view that the partial degradation or diminution of the cytoplasm might be part of the process of preparing specimens or a sign of the degenerative spectrum of entire epithelial cells.

As is already well known, the most striking characteristics in bacterial gill disease are hyperplasia of gill epithelia and fusion of adjacent gill lamellae (Wood and Yasutake, 1957; Amlacker, 1970; Wolke, 1975). Although Wood and Yasutake (1957) reported that the bacterial type of hyperplasia frequently developed in the lamellar epithelium and was often detected first at the extreme distal tip of the lamellae, the present observations and our other data on experimental infection of bacterial cells (Kudo and Kimura, 1983) have revealed that hyperplasia starts at the distal end of the filaments and progresses towards the proximal portion. This may be closely related to the location of the first adhesion of bacterial cells. Hypertrophy of the lamellae, which was frequently observed in the comparatively wide region of advancing lesions, began at the extreme distal tip of the gill lamellae and progressed towards the filament with no proliferation of epithelial and chloride cells. The direction of this progression may be related to the first adhesion of the bacterial cells to the tips of the gill lamellae. On the other hand, the complete fusion of gill lamellae can be classified into two processes. One is that the fusion starts at adjacent lamellar tips to form an interlamellar cavity between the two lamellae, and the concomitant proliferation of cells in the interlamellar epithelium results in the complete closure of the cavity. This conjecture is well-founded, due to the presence of degenerating chloride and mucous cells enclosed in the deep interior of the lesion. The other process may be that proliferation of interlamellar (or filament) epithelial cells progresses in a direction to fill the region between two adjacent lamellae. This can be presumed from the fact that chloride cells were often situated only in the outermost layer of hyperplastic lesions from which parts of adjacent lamellar tips projected without fusion. The arrangement of epithelial cells has given no evidence that they induce the entire fusion of adjacent lamellae without their proliferation, but it is unclear whether the same factor induced both phenomena — the fusion of lamellae and the proliferation of epithelial cells.

The existence of "plaques" projecting from the lamellae has been reported as one of the developmental features in bacterial gill disease (Rucker et al., 1952; Wood and Yasutake, 1957; Kimura et al., 1978). Identical globoid structures have generally contained various inclusions originating from degenerating lamellar epithelial and/or chloride cells and occasionally macrophage, with some empty exceptions. The empty globoids are probably the last step in the globoid formation process and seem to have been formed by part(s) of the peripheral cytoplasm or flattened perikaryon(a) which was left after clearance of the degenerating cytoplasm or cell debris by macrophages. In more advanced lesions, neither globoids nor separate lamellae have been found. Instead, there appeared many layers of flat or spindle-like epithelial cells which had a good resemblance structurally to the cells forming the globoid structures. These suggest that the flat cells forming these structures may have also been involved in the pilling-up of the superficial layers of flat or spindle-like epithelial cells in the club-like hyperplasia, together with other epithelial cells proliferated in the lamellar and filament epithelia.

It has recently been reported that plasma membrane vesiculation results from cell injury which blocks cellular metabolism and growth (Scott, 1976; Hoerl and Scott, 1978). RR staining revealed that plasma membrane vesiculation or blebbing was more striking in cells with darker cytoplasm, whose darkness was perhaps caused by the diffusion of the dye into the cytoplasm through the plasma membrane, because

normal cells allow little such diffusion. Thus, the membrane blebbing may have been closely associated with the process of cell injury caused by some factor originating from the bacterium transmitting bacterial gill disease.

Lymphocyte-like cells which appeared in more advanced or the most serious lesions exhibited not only an ultrastructural cytoplasmic texture similar to epithelial or chloride cells but also occasionally cytoplasmic continuity with some of the cell debris around them. Therefore, the lymphocyte-like cells might originate from the epithelial or chloride cells which had already lost the greater part of their cytoplasm due to degeneration. There is no evidence, however, of whether the lymphocyte-like cells are only one of the spectra in the degenerating process of epithelial or chloride cells or in the process of transformation into flat cells, or whether they are destined to be phagocytized by macrophages. Further, it is worth notice that lymphocyte-like cells adjoining the erythrocytes enclosed in advanced or severe lesions had similarities with pillar cell ultrastructure. The fate of pillar cells could not be clarified in the present investigation, but it seems important to learn whether or not they are closely connected with unusual cells in the blood capillary spaces surrounded by pillar cell flanges or lymphocyte-like cells. This question remains to be clarified by further investigation. On the other hand, the meaning of epithelial cell exfoliation in advanced or serious hyperplastic lesions is at present obscure, that is, whether or not the exfoliation has some similarity to that of superficial kelatinized cells of the skin, although the morphology of exfoliating cells in bacterial gill disease is completely different from the kelatinized cells.

In addition to epithelial hyperplasia in bacterial gill disease, it is well known that hyperplasia of gill epithelium results from a continuous exposure to various irritants such as ammonium hydroxide (Eller, 1975), heavy metals (Amend and Yasutake, 1969; Bilinski and Jonas, 1973; Wobeser, 1975a, b), pesticides (Eller, 1975; Walsh and Ribelin, 1975), crude oil or its water-soluble fractions (Solangi and Overstreet, 1982), and from the infection with protozoa (Sawyer et al., 1975) or with fungi (Eller, 1975). Further, pantothenic acid deficiency also causes the epithelial hyperplasia of secondary lamellae in salmonids (Rucker et al., 1952; Halver, 1969; Snieszko, 1972; Eller, 1975). From morphological viewpoints, hyperplasia in the gill epithelium has some similarities in proliferation of epithelial cells and in progression of the lesion in spite of the differences in various irritants. This suggests that response of the epithelial cells to irritants may result in a morphologically similar representation. Although this response is possibly one of defensive mechanisms, its precise significance of increased cellular proliferation has to be elucidated by further investigations.

Most investigators believe bacterial gill disease to be principally a myxobacterial infection (Amlacher, 1970; Eller, 1975). However, we believe the disease is caused by the infection of *Flavobacterium* sp. which has been isolated by us (Kimura et al., 1978), and we have succeeded not only in reproduction of the disease using the bacterium (Kudo and Kimura, 1983) but also in extraction of a material inducing the disease from the bacterium (Kudo and Kimura, in press b).

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透過型電子顕微鏡によるニジマス稚魚の細菌性鰓病に関する研究*

工藤重治・木村紀彦

細菌性鰓病の初期病巣は超微形態学的には二次鰓弁の肥厚によって始まる．その上皮細胞は立方または円柱状，塩類細胞は円形または卵円形である．しかし，粘液細胞の形は識別できるほどの変化はない．これらの細胞間には大食細胞や好中球の浸潤がみられる．また，上皮細胞，塩類細胞および粘液細胞の自由表面では形質膜の小胞化がよくみられる．二次鰓弁の表面には球状の突出物がしばしば現われるが，これはプラークに相当するものと思われる．これは肥大した上皮細胞や塩類細胞の細胞質が変性し，最終的には周縁の細胞質と核のみが扁平状に残り，しかもその変性した細胞質は大食細胞によって貪食され，内部が空胞(液胞)状に膨化して出来たものである．これらの球状体の形成には 1～数個の細胞が関与している．

* ニジマス稚魚の細菌性鰓病に関する超微形態学的研究—I

病巣の進んだところ，すなわち過形成の部位では二次鰓弁や鰓弁の癒合，上皮細胞の分裂像，病巣内部に閉じ込められて変性しつつある上皮細胞や塩類細胞の周縁細胞質の除去，游走細胞の浸潤および柱細胞の形態学的変化がみられる．

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