Different Patterns of Acetylation and Dimethylation of Histone H3 between Young and Aged Cases with Chronic Tonsillitis: Influences of Inflammation and Aging

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Introduction: Epigenetics is now considered to be crucially involved in normal genetics and differentiation and in pathological conditions, such as cancer, aging, and inflammation. Epigenetic mechanisms involve DNA methylation and histone modifications. The purpose of this study was to investigate the effects of inflammation on epigenetics in young subjects and the effect of aging.

Materials and Methods: The palatine tonsils were extracted from child and adult patients with chronic tonsillitis. Hematoxylin-eosin staining was performed to examine the morphology of the palatine tonsils. A fluorescence immunological examination was also performed to detect acetyl-histone H3 or dimethyl-histone H3. Confocal scanning microscopy was used for observations.

Results: Acetylated histone H3 was detected in tonsils from child patients but not from adult patients. Dimethylated histone H3 was not detected in tonsils from either group of patients. Degeneration of the tonsillar structures was apparent in tonsils from adult patients.

Discussion: The differential expression of acetylated histone H3 Lys9 may reflect immunological differences between young and aged tonsils. The decrease observed in the activity of histone methyltransferase induced the down-regulated expression of methylated histone H3.

Conclusion: Our results suggest that epigenetic changes participate in chronic inflammation and aging in the palatine tonsils. Although the results do not lead to a direct treatment, the epigenetic pathogenesis of chronic inflammation, such as immunoglobulin A nephropathy, by focal infections will be described in greater detail in future studies, which will lead to new treatments being developed.

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Key words: epigenetics, histone modification, chronic tonsillitis, acetylation, dimethylation
performed tonsillectomy for patients with chronic tonsillitis. The etiology of age varied from young to aged patients.

The purpose of the present study was to investigate histone modification patterns under inflammation, particularly in young patients, and to determine the effects of aging on these patterns.

Materials and Methods

Palatine tonsils were extracted from 8 patients (6 male and 2 female) with chronic tonsillitis. All patients had previously had 6 or more episodes of tonsillitis per year or 3 or more episodes in the 2 years preceding this study and underwent tonsillectomy in our hospital in 2012 or 2013. Tonsillectomy was performed with the patient under general anesthesia. The tonsils were grasped with a clamp, the tonsillar capsule was separated from the tonsillar bed, and the tonsils were then extracted. Patients (Table 1) were divided into 2 groups: a child group (n=4) aged 2 to 6 years (mean age±SD, 3±2 years) and an adult group (n=4) aged 37 to 46 years (mean age, 40±4.2 years). Patients did not have focal infections, such as immunoglobulin A nephropathy and palmoplantar cystic disease.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Chief complain</th>
<th>Clinical course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>female</td>
<td>fever, sore throat</td>
<td>chief complaint recurs</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>female</td>
<td>fever, sore throat</td>
<td>chief complaint recurs</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>male</td>
<td>fever</td>
<td>chief complaint recurs</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>male</td>
<td>fever</td>
<td>chief complaint recurs</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>male</td>
<td>fever, sore throat, dysphagia</td>
<td>chief complaint recurs</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>male</td>
<td>fever, sore throat</td>
<td>chief complaint recurs</td>
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<td>7</td>
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<td>male</td>
<td>fever, sore throat</td>
<td>chief complaint recurs</td>
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<tr>
<td>8</td>
<td>37</td>
<td>male</td>
<td>fever, sore throat</td>
<td>chief complaint recurs</td>
</tr>
</tbody>
</table>

The palatine tonsils of 8 patients (6 male and 2 female) with chronic tonsillitis were extracted. The main symptom was fever. None of our patients had focal infections, such as immunoglobulin A nephropathy and palmoplantar cystic disease.
**Results**

**Morphological Changes Revealed by Hematoxylin and Eosin Staining in Human Patients**

The tonsils of patient 2, a child, presented a mature structure, in which the germinal center and a large number of lymphocytes were detected (Fig. 1a). The same mature structure was observed in the tonsils from patient 3 (Fig. 1b). The structure of the tonsils presented the same features in all patients who were children.

The degeneration of lymphoid tissues and fatty changes were observed in the tonsils of patient 5, an adult (Fig. 1c). The degeneration of the germinal center was also detected in patient 6 (Fig. 1d). Degenerative changes in the germinal center were a common feature in the tonsils from all patients who were adults.

**Immunohistochemical Study for CD4**

Palatine tonsil tissues in the young group showed positive stainings for CD4. CD-positive cells (T-cells) were scattered in the germinal center and surrounding areas (Fig. 2a, b). In the older group, T-cells were also...
Histone Modification in Cases with Chronic Tonsillitis

Fluorescence Immunohistochemical Expression of Acetylated and Dimethylated Histones

Acetylated histone H3 lysine 9 (Lys9) was detected in the nuclei of the tonsillar germinal center in young group (Fig. 3a), whereas dimethylated histone H3 Lys9 was not (Fig. 3b). These immunoreactivities were not observed in the area surrounding the germinal center or epithelium.

Acetylated histone H3 Lys9 and dimethylated histone H3 Lys9 were not detected in the nuclei of the germinal centers in the old group (Fig. 3c, d).

The organ of Corti in young mice showed positive staining for acetylated histone H3 Lys9 (Fig. 3e), whereas that in old mice showed positive staining for dimethylated histone H3 Lys9 (Fig. 3f). Negative staining was observed in the young group for dimethylated histone H3 Lys9 (Fig. 3g) and in old mice for acetylated histone H3 Lys9 (Fig. 3h).

Discussion

In the present study, observations with a light microscope revealed that the structure of the palatine tonsils was mature in child patients, whereas that in adult patients had degenerated. A previous study has demonstrated that the structure of the palatine tonsils is fully developed in children younger than 10 years\textsuperscript{12}. Active nodules and the infiltration of dense lymphocytes have previously been reported. Furthermore, degeneration of the tonsils and the appearance of adipose tissues occurred after the age of 21 years. Our morphological results are consistent with previous findings.

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Detected, particularly in the area surrounding the germinal center. Some atrophic changes were observed around the germinal center (Fig. 2c, d).

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Fig. 3  Six-micrometer-thick paraffin sections of palatine tonsils. The distribution of acetylated H3 Lys9 or dimethylated H3 Lys9, DNA stained with DAPI and DRAQ5 (blue), acetylated H3 Lys9 and dimethylated H3 Lys9 stained by immunofluorescence using a Cy3-conjugated antibody (red).

(a) Palatine tonsil tissues in the young group.
Acetylated H3 Lys9 was detected in the nuclei of germinal center cells (arrow).
(b) Palatine tonsil tissues in the young group.
Dimethyl H3 Lys9 was not detected.
(c) Palatine tonsil tissues in the old group.
Acetylated H3 Lys9 was not detected.
(d) Palatine tonsil tissues in the old group.
Dimethyl H3 Lys9 was not detected.
(e) The organ of Corti in young mice (C57BL/6) at the age of 8 weeks.
Positive staining for acetylated histone H3 Lys9 was observed (arrow).
(f) The organ of Corti in old mice (C57BL/6) at the age of 132 weeks.
Positive staining for dimethylated histone H3 Lys9 was observed (arrow).
(g) The organ of Corti in young mice
No staining for dimethylated histone H3 Lys9 was observed.
(h) The organ of Corti in old mice
No staining for acetylated histone H3 Lys9 was observed.
Histone Modification in Cases with Chronic Tonsillitis

Fig. 4  Schematic drawing of histone modifications under inflammatory and aged conditions.
Inflammatory factors trigger the activation of histone acetyl transferase and histone demethylase.
Aging reflects many factors, such as environmental factors, recurrent inflammation, and genetic factors. These influences increase the activity of histone deacetylase and histone demethylase. The suppression of acetylated histones induces apoptosis.

Pharynx, whereas lingual tonsil adenoids are located at the entrance to the oropharynx, which is called Waldeyer’s ring. The surface of the tonsils is covered by a squamous epithelium. Crypts enter the tonsils. Many nodules display the infiltration of lymphocytes; these nodules contain the germinal centers. The palatine tonsils function as an immune barrier against unwanted organisms and antigens and are immunologically active between the ages of 4 and 10 years. The palatine tonsils contain T cells, macrophages, dendritic cells, and B cells, which account for 50% to 65% of all tonsillar lymphocytes in the germinal centers. Information regarding the infiltration of an antigen is relayed to mature T cells and B cells to start the production of antibodies against it. The B cell population decreases after puberty, when the ratio of T to B cells increases. Immunological activity appears to decrease after the age of 21 years.

In the present study, acetylated histone H3 Lys9 was detected in the germinal centers of the tonsillar tissues in young mice, whereas dimethylated histone H3 Lys9 was not. In contrast, acetylated histone H3 Lys9 and dimethylated histone H3 Lys9 were not detected in old mice.

Chronic tonsillitis is characterized by the permanent inflammation of the palatine tonsils as a result of recurrent acute or subclinical infections. The hypermethylation of DNA has been detected with chronic inflammation, such as atherosclerosis. Epigenetic mechanisms have been shown to modulate the expression of the proinflammatory cytokine, tumor necrosis factor-α, interleukins, and tumor suppression genes under chronic inflammatory conditions. Chronic obstructive pulmonary disease is a chronic inflammatory disease of the lung, and in patients with this disease the expression of histone deacetyltransferase is down-regulated. Levels of histone acetylation are high in proinflammatory genes. Immune responses are enhanced by Jumonji D3 domain-containing protein 3, which is a histone demethylase. Histone methyltransferase regulates chromatin and suppresses its expression in pulmonary diseases. This is a reason that methylation is suppressed. Elevated levels of acetylated histones are known to contribute to inflammation. The immunological activity of the palatine tonsils was previously reported to be the highest in young children. Taken together, these findings support the expression of acetylated histones and suppression of dimethylated histones in the palatine tonsils in the young subjects of our study.

A previous study has demonstrated that the immu-
nological responses of the palatine tonsils decrease after the age of 21 years\textsuperscript{2}. The differential expression of acetylated histone H3 may reflect the immunological differences observed between the younger and older subjects. On the other hand, monozygotic twins show an altered pattern of epigenetic modification, that is, various factors are involved in the DNA methylation and histone modification\textsuperscript{3}. Histone deacetyltransferase plays key roles in cellular aging\textsuperscript{4}. Aging also leads to a decrease in histone acetylation, which results in reduced memory activity\textsuperscript{5} and induced apoptosis\textsuperscript{6}. Histone demethyltransferase has been shown to trigger the aging process\textsuperscript{7}. In the present study, we did not detect acetylated or dimethylated histone H3 in the older subjects. Therefore, aged tonsillar tissue may be influenced by inflammation and aging. The acetylation of histones gradually decreases during the process of aging. We summarize the relationships of inflammation, aging, and histone modifications in Figure 4. Complications associated with chronic tonsillitis in young and aged patients include peritonsillar and parapharyngeal abscesses, recurrent high fever, and glomerulonephritis\textsuperscript{8}. The radical treatment currently employed for chronic tonsillitis is tonsillectomy. Abnormalities have been detected in DNA methylation and histone modifications in cancer cells. A histone deacetyl transferase inhibitor has recently been clinically applied to the treatment of leukemia\textsuperscript{9}. For ethical reasons, we did not compare the tissue examined with normal tonsils. Although the results of the present study do not lead to a direct treatment, the epigenetic pathogenesis of chronic inflammation, such as immunoglobulin A nephropathy, by focal infections will be described in greater detail in future studies, which will lead to new treatments being developed.

In conclusion, acetylated histone H3 was detected in the germinal centers of palatine tonsils in young subject but not in the older subjects. Dimethylated histone H3 was not detected in either group of subjects. These modification patterns of histones were presumed to reflect the influences of inflammation and aging.

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References


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