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Original Article

Epithelioid Cell Granulomatous Response of Waldeyer's Ring among Japanese : A Clinicopathological and Immunohistochemical Study of 16 Cases

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The tonsils are uncommonly affected by granulomatous inflammation. This study attempted to clarify the clinicopathological and immunohistochemical findings and presence or absence of Epstein-Barr virus (EBV) in tonsilar granulomatous inflammation. A total of 537 consecutive specimens from tonsillectomies performed at Dokkyo University School of Medicine between 1999 and March 2012 were reexamined. Using formalin-fixed, paraffin-embedded sections, histological, immunohistochemical, and *in situ* hybridization (ISH) studies were performed. Epithelioid granulomas (EPGs) were identified in the tonsils in 16 (3.0%) cases. There were 8 males and 8 females, aged 4 to 57 years (mean, 23). In 11 patients, EPGs were located in the germinal center (GC), whereas they were located in the interfollicular area as well as GC in the remaining 5 cases. Three types of EPG have been delineated : (i) poorly demarcated small epithelioid cell granulomas (n = 6) ; (ii) well-demarcated non-caseating sarcoid-like granulomas (n = 5) ; and (iii) EPGs within GC showing suppurations at the center (n = 5). An ISH study demonstrated EBV-encoded small RNA (EBER)$^+$ cells in 4 lesions. The present study demonstrated that the majority of EPGs were located in the GC and tonsilar EPGs showed histological variation. 〔*J Clin Exp Hematopathol 52(3) : 179-184, 2012*〕

Keywords: epithelioid cell response, Waldeyer's ring, clinicopathologic study, immunohistochemistry, germinal center

INTRODUCTION

Waldeyer's ring is rarely affected by granulomatous inflammation, but, when present, is occasionally part of systemic diseases such as sarcoidosis, Crohn's disease, fungal infection, and tuberculosis.[1-4] Kardon and Thompson reported that follicular hyperplastic lymphoid follicles showed the replacement of germinal centers (GCs) by well-formed epithelioid cell granulomas (EPGs) in chronic tonsillitis.[2,4] However, little is known about the clinicopathological and immunohistochemical findings regarding the epithelioid granulomatous response in tonsils.[2] To clarify tonsilar epithelioid cell granulomatous inflammation in Japan, we conducted clinicopathologic, immunohistochemical, and *in situ* hybridization (ISH) analyses on 16 such cases in the Northern Kanto district.

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PATIENTS AND METHODS

This study was based on 537 consecutive specimens from tonsillectomy performed in the Dokkyo University School of Medicine between 1999 and March 2012. Clinical findings were obtained from hospital records. One section per tonsil was available. Three-μm-thick sections were cut from formalin-fixed, paraffin-embedded tissues, and stained with hematoxylin-eosin and Gram stain. If necessary, Giemsa, periodic acid-Schiff (PAS), and Ziehl-Neelsen stains were performed.

EPGs were categorized according to Kardon and Thompson,[2] including (i) the distribution (focal, scattered, or diffuse) ; (ii) location (within GC or interfollicular (IF)) ; (iii) circumscription (well circumscribed or poorly circumscribed) ; (iv) size (small, medium, or large) ; and (v) necrosis (present or absent).

Immunohistochemistry was performed on paraffin sections using a Histofine Histostainer (Nichirei Bioscience Inc., Tokyo, Japan) according to the manufacturer's directions. A basic panel of antibodies against human PS-1 (CD3 ; Nichirei Bioscience Inc., Tokyo Japan), 7 (CD14 ; Leica Biosystems, Newcastle, UK), L26 (CD20 ; Nichirei Bioscience Inc.), SP23 (CD23 ; Nichirei Bioscience, Inc.), 1B16 (CD56 ; Nichirei Bioscience Inc.), KP-1 (CD68 ; Dako A/S, Glostrup, Denmark), LN22 (bcl-6 ; Leica Biosystems), and AF801

(*Toxoplasma gondii*; R&D Systems, Minneapolis, Minnesota, USA) were applied in all 16 cases. If necessary, immunohistochemical analysis was expanded to include antibodies IgM (Nichirei Bioscience Inc.), IgD (Nichirei Bioscience Inc.), AE1/3 (cytokeratin; Nichirei Bioscience Inc.), and H2A10 (*Bartonella henselae*; Biocare Medical, Concord, CA, USA). Sections with known reactivity for the antibodies assayed served as positive controls and sections treated with normal mouse serum served as negative controls. ISH with Epstein-Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBV small RNA in formalin-fixed, paraffin-embedded sections using a hybridization kit (Dako Y5200 and K5201).

RESULTS

Incidence of the epithelioid granulomatous response

EPGs were identified in the tonsils in 16 (3.0%) of 537 cases, and their clinicopathological findings are summarized in Table 1.

Clinical findings

Eight were men and 8 were women, with an age range of 4 to 57 years (mean, 23.1). The tonsils were involved in 15 (nos. 2-16) cases and the adenoids in one (no. 1). Most of the patients presented a bilateral tonsilar mass with a fever and/or sore throat. In 2 cases (nos. 11 and 16), EPGs affected the bilateral tonsils, whereas they affected the unilateral tonsils in the remaining 14 (nos. 1-10, 12-15). There was no history of sarcoidosis, tuberculosis, or Crohn's disease in any of the 16 cases. One case each had a history of infectious mononucleosis (IM) 14 (no. 4) and 24 (no. 8) months before tonsillectomy. Two cases (nos. 10 and 16) had a history of bronchial asthma, and 2 (nos. 2 and 5) had a history of allergy to house dust. Bacterial culture obtained from tonsilar pus demonstrated α-streptococci in 1 case (no. 10).

Histopathological, immunohistochemical, and EBV analyses of EPGs

Histologically, EPGs were scattered in 13 cases (nos. 3-12, 14-16) and focal in only 3 cases (nos. 1, 2, and 13). EPGs were located within GC in 11 cases (nos. 2-4, 6-10, 12, 14, and 16) and within both GC and IF in 5 cases (nos. 1, 5, 11, 13, and 15) (Fig. 1).

Three types of EPGs have been delineated: (i) poorly demarcated small epithelioid cell granulomas (n = 6; nos. 1, 2, 5, 8, 13, and 16) (Fig. 2); (ii) well-demarcated medium and/or large non-caseating sarcoid-like granulomas (n = 5; nos. 4, 6, 11, 14, and 15) (Fig. 1) (On a high-power field, epithelioid cell granulomas contained small lymphocytes;

Table 1. Sammary of 16 cases

No.	Age	Gender	Site	Symptom	Clinical diagnosis	Allergy	Distribution	Location	Demarcation	Size	Necrosis	Gram	EBER
1	4	M	Adenoid	Mass	Sleep apnea	−	Focal	GC + IF	Poorly	Small	−	−	−
2	6	M	Rt. tonsil	Mass	Sleep apnea, habitual angina	House dust	Focal	GC	Poorly	Small	−	−	−
3	6	M	Lt. tonsil	Deafness, mass	Habitual angina, otitis media	−	Scattered	GC	Well	Large	Abscess	P, Cocci	−
4	7	M	Rt. tonsil	Fever, mass	Habitual angina	−	Scattered	GC	Well	Medium	−	P, Cocci	+
5	8	F	Lt. tonsil	Mass	Sleep apnea	House dust	Scattered	GC + IF	Poorly	Small	−	−	−
6	19	F	Rt. tonsil	Fever, sore throat, mass	Chronic tonsiltis	−	Scattered	GC	Well	Medium to large	−	P, Cocci	−
7	19	F	Lt. tonsil	Fever, sore throat, mass	Habitual angina	−	Scattered	GC	Well	Large	Abscess	P, Cocci	+
8	20	M	Lt. tonsil	Mass	Sleep apnea	−	Scattered	GC	Poorly	Small	−	−	+
9	25	M	Rt. tonsil	Fever, sore throat, mass	Habitual angina	−	Scattered	GC	Well	Large	Abscess	−	−
10	26	F	Lt. tonsil	Fever, sore throat, mass	Habitual angina	Bronchial asthma	Scattered	GC	Well	Large	Abscess	−	−
11	26	F	Bil. tonsil	Soret throat, mass	Chronic tonsiltis, otitis media	−	Scattered	GC + IF	Well	Medium to large	−	−	−
12	27	F	Lt. tonsil	Fever, sore throat, mass	Habitual angina	−	Scattered	GC	Well	Large	Abscess	P, Cocci	−
13	30	M	Rt. tonsil	−	IgA nephropathy	−	Focal	GC + IF	Poorly	Small	−	P, Cocci	−
14	35	M	Lt. tonsil	Mass	Sleep apnea	−	Scattered	GC	Well	Medium to large	−	−	−
15	54	F	Lt. tonsil	Sore throat, mass	Chronic tonsiltis, otitis media	−	Scattered	GC + IF	Well	Medium to large	−	P, Cocci	−
16	57	F	Bil. tonsil	Sore throat, mass	Chronic tonsiltis	Bronchial asthma	Scattered	GC	Poorly	Small	−	−	+

Rt, right; Lt., left; Bil., bilateral; GC, germinal center; IF, interfollicular; EBER, Epstein-Barr virus-encoded small RNA; P, positive.

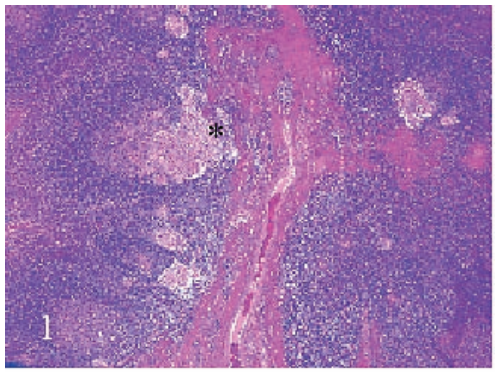

Fig. 1. On low-power magnification, medium- and large-sized, well-circumscribed epithelioid granulomas were located within germinal centers (*) and the interfollicular area. Case 15, H&E, x10.

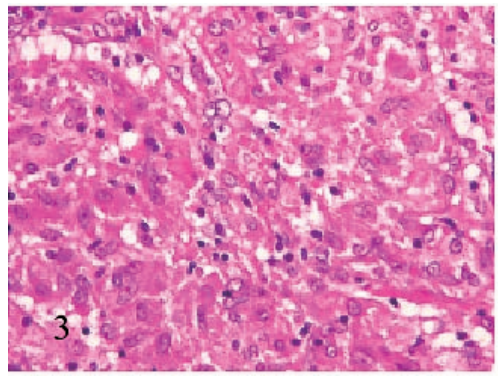

Fig. 3. High-power magnification of Fig. 1. A large epithelioid granuloma contained small lymphocytes. H&E, x100.

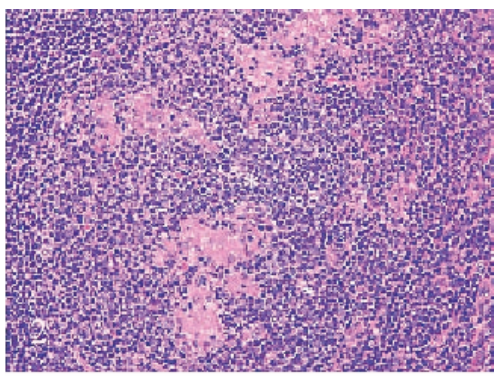

Fig. 2. On medium-power magnification, poorly circumscribed small epithelioid granulomas were located within a germinal center (*). Case 6, H&E, x50.

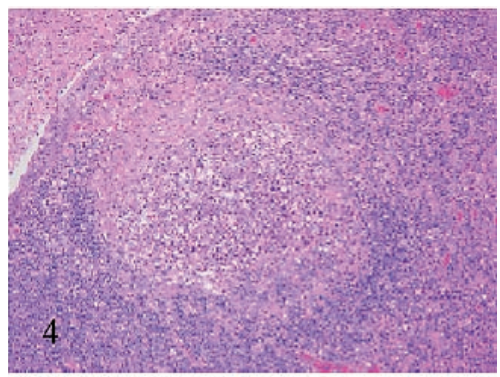

Fig. 4. On medium-power magnification, well-developed epithelioid granulomas within the germinal center showed suppurations at the centers. Case 9, H&E, x50.

Fig. 3) ; and (iii) large, well-demarcated EPG within GC showing suppurations at the center (n = 5 ; nos. 3, 7, 9, 10, and 12) (Fig. 4). In the central suppurations, scattered centrocytes and centroblasts were present (Fig. 5). EPGs did not contain monocytoid B cells (MBCs) in any of the 16 lesions.

An immunohistochemical study demonstrated histiocytes with the feature of $CD14^+$, predominantly composed of $CD14^+$ (Fig. 6), and $CD68^+$ histiocytes with or without epithelioid cell features. Within the EPGs, the majority of the small lymphocytes were $CD3^+$ with a few $CD20^+$ B cells. $CD56^+$ cells were absent in the lesion. CD23 immunostaining demonstrated a follicular dendritic cell (FDC) network. Occasionally, both non-suppurative and suppurative EPGs contained a residual FDC network (Fig. 7). Moreover, CD20 and bcl-6 immunostaining demonstrated residual GC cells in both non-suppurative and suppurative EPGs (Fig. 8). Lymphoid cuffs of small lymphocytes surrounding the EPGs within GC were usually $CD20^+$, surface IgM/D^+ mantle zone lymphocytes.

There were gram-positive cocci at the crypt epithelium of the tonsil in 7 cases (nos. 3, 4, 6, 7, 12, 13, and 15) showing suppurative granulomas (Fig. 9). An immunohistochemical study demonstrated that there was no *Toxoplasma gondii* in any of the 16 lesions. Acid-fast bacilli, fungus, and *Bartonella henselae* were absent in 5 cases with suppurative granulomas (nos. 3, 7, 9, 10, and 12).

On ISH, a positive signal for EBERs was identified in 4 cases (nos. 4, 7, 8, and 16). More than 500 $EBER^+$ cells were located in the GCs including microabscess as well as IF areas in 1 case (no. 7; Fig. 10). Two follicular GCs with EPGs contained $EBER^+$ cells as well as IF areas in another case (no. 4 ; Fig. 11). Up to 10 $EBER^+$ cells were located in the IF area and/or GC in the remaining 2 cases (nos. 8 and 16).

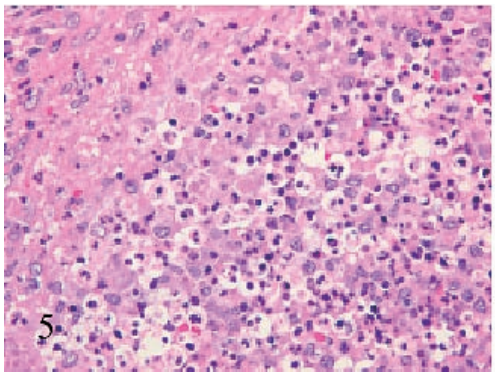

Fig. 5. High-power magnification of Fig. 4. Note the scattered centrocytes and centroblasts in the central suppurations. Case 6, H&E, x100.

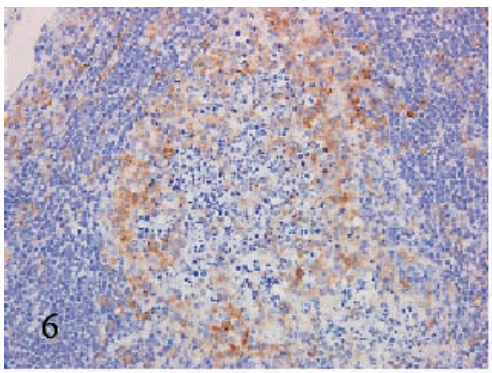

Fig. 6. CD14 immunostaining demonstrated histiocytes with or without epithelioid cell features. Case 9, x50.

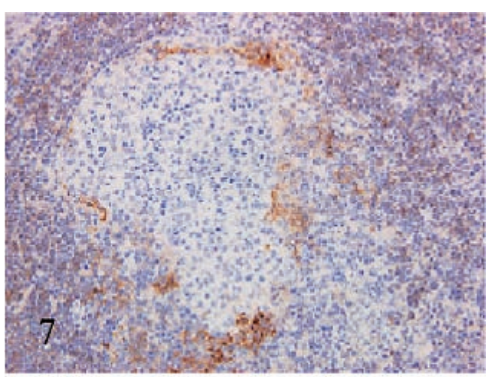

Fig. 7. CD23 immunostaining demonstrated residual follicular dendritic cell network within an epithelioid granuloma having central suppression. Case 9, x50.

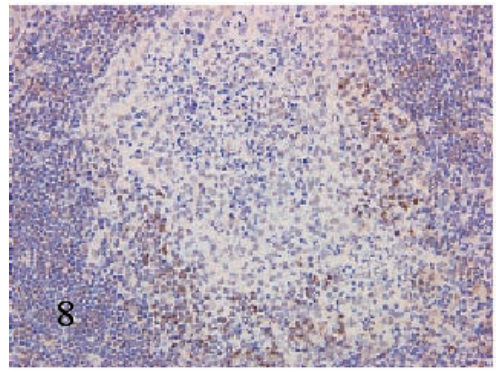

Fig. 8. Bcl-6 immunostaining demonstrated residual follicular dendritic cell network within an epithelioid granuloma having central suppression. Case 9, x50.

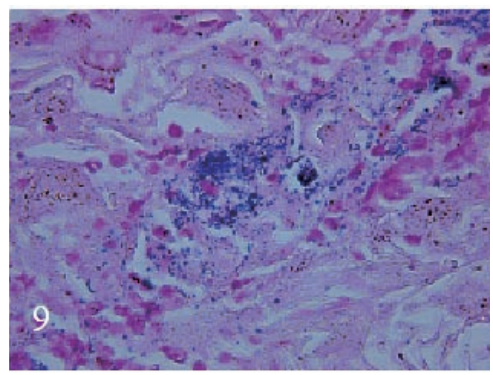

Fig. 9. There were gram-positive cocci at the crypt epithelium of the tonsil. Case 7, Gram stain, x150.

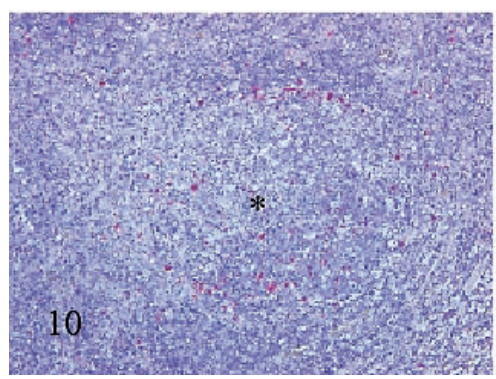

Fig. 10. There were numerous Epstein-Barr virus-encoded small RNA-positive cells in a germinal center including a microabscess (*) as well as the interfollicular area. Case 7, x25.

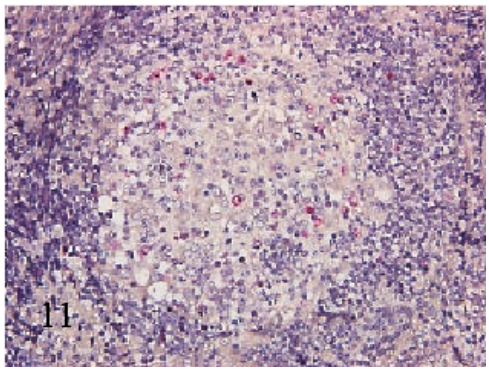

Fig. 11. There were numerous Epstein-Barr virus-encoded small RNA-positive cells within epithelioid granulomas in a GC. Case 4, x25.

DISCUSSION

Compared with the U.S.A. (0.08%), tonsilar EPGs in Japan appear to show higher incidence (0.3%).[2] In the U.S.A., the majority of tonsilar lesions were obtained from military sources, whereas in Japan, most of the tonsilar lesions were from civilians. The cause of the difference in incidence of tonsilar EPGs between the U.S.A. and Japan appears to be the population source. Moreover, only one section per tonsil was available in our study. Small focal EPGs may have been overlooked in this study.

In this study, three types of EPGs are delineated : (i) Poorly demarcated small epithelioid cell granulomas (n = 6). This type of EPG is somewhat similar to EPGs in toxoplasmic lymphadenitis.[5] An immunohistochemical study demonstrated no *Toxoplasma gondii* in any of the 6 lesions of this type of EPG. However, no serological data for *Toxoplasma gondii* were available in any of the 6 cases. (ii) Well-demarcated medium and/or large non-caseating sarcoid-like granulomas (n = 5) : This type of EPG is similar to sarcoid granuloma. Interestingly, the presence of non-caseating EPGs in the GCs is a characteristic histological finding of lymph node lesions in Crohn's disease.[6,7] However, there was no history of sarcoidosis, tuberculosis, or Crohn's disease in any of the 5 cases. (iii) Well-demarcated large EPGs within GC showing suppurations at the center (n = 5) : On the basis of the histological and immunohistochemical features, suppurative EPGs were classified into two subtypes : B-cell$^-$ and B-cell$^+$ granulomas.[8-11] The former type of suppurative EPG was composed of histiocytes with or without epithelioid cell features and scattered T-lymphocytes.[7,10-12] The former type of suppurative EPG was located in the GC and IF area.[7,10-12] The former type of suppurative EPG was caused by *Yersinia. enterocolitica*.[7,10-12] The latter type of suppurative EPG contained MBCs as well as histiocytes and scattered T-lymphocytes.[9-11] EPGs were usually located in the IF area because of the parafollicular distribution pattern of the MBCs.[9-11] The latter types of suppurative EPG were observed in cat scratch disease, lymphogranuloma venereum, and tularemia lymphadenitis.[9-11] In our 5 EPGs having central suppurations, there were scattered B cells among numerous neutrophils. However, bcl-6 immunostain demonstrated that these B cells were residual GC B cells in the microabscess. The present study indicated that suppurative tonsilar EPGs appear as B-cell-negative EPGs. Immunohistochemical study demonstrated there was no *Bartonella henselae* in any of the 5 lesions.[13] There were gram-positive cocci at the crypt epithelium of the tonsil in 7 cases. Moreover, bacterial culture obtained from tonsilar pus demonstrated α-streptococci cocci in 1 case. However, gram-positive cocci including α-streptococci are not usually causative agents of granulomatous inflammation.[10] The causative agents of tonsilar EPGs having central suppurations remain unclear.

EBER$^+$ cells were identified in 4 cases. Among them, 2 had a history of IM. More than 500 EBER$^+$ cells were located in the GCs including microabscess as well as IF area in 1 case. Two follicular GCs with EPGs contained EBER$^+$ cells in another case. Recently, EBER$^+$ cells have usually been reported in the IF area.[14-16] Kurth *et al*. concluded that the presence of numerous EBER$^+$ cells in the GCs as well as the IF area appears to be a characteristic finding of recent EBV infection (infectious mononucleosis pattern).[16] These findings indicate that EBV may be another causative agent of tonsilar EPGs. However, neither of the 2 patients exhibited atypical lymphocytosis in the peripheral blood. Moreover, anti-EBV antibodies were not examined in either of these 2 cases.

Clinically, the mean age of patients with poorly demarcated small epithelioid cell granulomas and well-demarcated large EPGs within GC having suppurations at the center was 21 years. In comparison with the other 2 types of EPGs, the patients having well-demarcated medium and/or large non-caseating sarcoid-like granulomas were older (mean : 29 years). Interestingly, 4 of 6 cases having poorly demarcated small epithelioid cell granulomas had a history of sleep apnea syndrome. Moreover, 3 of 4 cases having a history of allergic disease showed poorly demarcated small epithelioid cell granulomas. However, there are too few cases to clarify the correlation between type of EPGs and clinical findings.

In conclusion, tonsilar EPGs appear not to be so rare a phenomenon and showed histological variation.

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