Inflammatory infiltrate of chronic periradicular lesions: an immunohistochemical study

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Abstract

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Aim To determine the cellular profile of human chronic periradicular lesions using immunohistochemical methods in order to study the differences in the cell infiltrate of periradicular granulomas and cysts.

Methodology The study population consisted of 45 individuals without any systemic disease. Biopsies were obtained during periradicular surgery. Paraffin-embedded sections were stained by the avidin–biotin complex method (ABC), whilst cryostat tissue sections were stained using the alkaline phosphatase antialkaline phosphatase assay (APAAP). These methods are highly valid and sensitive using a panel of specific monoclonal antibodies: CD4, CD8, CD3, CD10, HLADR, CD20, CD45RO, CD68 and CD57. The 45 specimens were characterized by the use of both techniques.

Results The 45 specimens were histologically diagnosed as: 25 periradicular granulomas, 17 periradicular cysts and 3 scar tissues. No statistically significant differences were detected in the inflammatory infiltrate between periradicular granulomas and cysts. Observation of the sections showed that the majority of inflammatory cells consisted of T and B lymphocytes and macrophages. T and B lymphocytes were equally distributed in 60% of the cases. The T_4/T_8 ratio ranged approximately from 1 to 3 and greater, being consistent with inflammation of periradicular tissues. The final differentiation of B lymphocytes to plasma cells was also detected, whilst natural killer (NK) cells were found in only 10 cases (22%). Moreover, antigen presenting cells and T suppressor/cytotoxic cells were found to be associated with both pre-existing and newly formed epithelium.

Conclusions Periradicular granulomas and cysts represent two different stages in the development of chronic periradicular pathosis as a normal result of the process of immune reactions that cannot be inhibited.

Keywords: immunohistochemistry, infiltrate, periradicular lesions.

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Introduction

The healthy dental pulp is protected from oral microorganisms by enamel and dentine. Exposure of the dental pulp to bacteria and their by-products, acting as antigens, may elicit nonspecific inflammatory responses as well as specific immunological reactions in the periradicular tissues (Trowbridge 1990, Stashenko *et al.* 1998). The periradicular lesions formed are the result of the local defense reactions against the bacterial challenge. Periradicular granulomas represent a subsequent reparative process of chronic local inflammation (Gao *et al.* 1988a), whilst periradicular cysts are thought to be derived from epithelial rests within or adjacent to granulomatous tissue (Neville *et al.* 1995).

The tissues of both cysts and granulomas are infiltrated by specific and nonspecific cells involved in the local immunological responses. The local antigen presentation taking place in periradicular tissues leads to activation of immune cells (Kaneko *et al.* 2001). Immunoactive cells produce soluble mediators for inflammatory responses, including antibodies and cytokines. The capacity for local antibody synthesis is contributory to antigen-specific immunity and may have a part in modulating the disease activity (Stern *et al.* 1981). Moreover, the cell distribution and the character of the lesions

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are probably regulated by the cytokines, which are the messenger molecules of these cells, this way regulating many cells' interrelationships (Kawashima & Stashenko 1999).

However, it has not been clarified whether immunopathological changes are caused directly by the cells or by the extension of their by-products, the cytokines (Torabinejad & Bakland 1978, Roitt *et al.* 1996). Inflammatory cell distribution in the periradicular tissues has not been identified completely, either in granulomas or in cysts. Therefore, various attempts have been made and many investigators have tried to obtain evidence by means of immunofluoresence (Nilsen *et al.* 1984, Lukic *et al.* 1990, Marton & Kiss 1993) or immunoenzymatic techniques (Kopp & Schwarting 1989, Piattelli *et al.* 1991, Marton & Kiss 1993, Tani *et al.* 1992, 1996).

In the present study, immunoenzymatic characterization of cells and molecules observed in the human chronic periradicular lesions was investigated. The specific aim was to determine the cellular profile of these lesions using immunohistochemical methods in order to study the differences in the cell infiltrate of periradicular granulomas and cysts.

Materials and methods

Tissues

Forty-five dental chronic periradicular lesions from 45 healthy patients, aged 17–30 were used in this investigation. The lesions were located around the apex of each tooth and were at least 5 mm in diameter, as measured on periapical radiographs. All specimens were randomly selected and obtained at the time of apicectomy or tooth extraction after curettage of the tissues. These patients had not taken any antibiotics for the last 3 months. According to the clinical data collected at the time, 40 specimens were derived from asymptomatic and chronic periradicular lesions, whilst the remainder were chronic but associated with symptoms.

Immunohistochemical methods

In the present study, two immunoenzymatic techniques were used: the alkaline phosphatase antialkaline phosphatase (APAAP) assay and the avidin–biotin complex (ABC) method (True 1990, Boenisch 1993).

After harvesting, each specimen was divided into two equal parts. Half of each specimen was snap-frozen in isopentan prechilled with nitrogen. The frozen specimens were then embedded in Tissue Tek II OCT compound, stored at -80°C and serially sectioned at 5 μm in a cryostat. The second half of each specimen was fixed in neutral buffered formalin (NBF), embedded routinely in paraffin wax and sectioned at 4 μm. At least one section of each specimen was stained with haematoxylin and eosin (H&E).

The cryostat sections were stained using APAAP assay, whilst paraffin-embedded sections were stained by the ABC method.

Monoclonal antibodies

A panel of monoclonal antibodies to antigens of cluster differentiation was used in this study. The sources and specificities of the antibodies are summarized in Table 1. Dilutions of the antibodies used are also given.

All sections were examined in a Nikon E600 Eclipse light microscope (Japan) and results were expressed as the mean count of cells per 10 high-power fields. Ten

Table 1 Monoclonal antibodies and sections used in this study

Antibody	Dilution	Clone	Specificity	Source
Cryo-section				
Anti-CD4	1/10	BL-TH4	Helper/inducer T cells	Monosan
Anti-CD8	1/10	MEM-31	Cytotoxic/supressor T cells	Monosan
Anti-CD3	1/10	CD3	All T cells	DAKO
Anti-CD10	1/10	MEM-78	CALLA cells	Monosan
Paraffin section				
Anti-κ	1/10	2B7	κ light chains of immunoglobulins	Monosan
Anti-λ	1/10	48	λ light chains of immunoglobulins	Monosan
Anti-HLADR	1/10	CR3/43	Antigen presenting cells	DAKO
Anti-CD20	1/80	L-26	B cells	DAKO
Anti-CD45RO	1/80	UCHL-1	Memory T cells (Pan-T)	DAKO
Anti-CD68	1/100	KP1	Macrophages	DAKO
Anti-CD57	1/20	HNK-1	Natural killer cells	Becton-Dickinson

Plasma cell counting (%)	Granulomas ($n = 25$) 55.55%	Cysts ($n = 17$) 37.77%	Scar tissues ($n = 3$) 6.66%	Total ($n = 45$) 100%
–	4	6	33	7
+	16	17		15
++	44	24	67	38
+++	32	29		29
++++	4	24		11

Table 2 Percentage of the plasma cell infiltration in the tissues examined

microscopic fields, representing the most dense cellular inflammatory infiltrate, were selected per specimen and positive cell numbers were estimated as a proportion of lesion area, using a point counting method (Alavi *et al*. 1998).

The scale corresponded to the percentage of the stained cells with the specific antibody each time, compared to the total cellular infiltration and counting was scored as following:

– No cells (negative).
+ Few cells (0–10%, weak).
++ Some cells (10–25%, moderate).
+++ Many cells (25–50%, intense).
++++ Plenty of cells, more than (50%, very intense).

Statistical analysis

All the data were collected, classified and entered into a spreadsheet for statistical analysis, using the Fisher's exact and the Wilcoxon exact tests. Periradicular granulomas and cysts were compared for the following variables and cell counts: CD4/CD8, CD3, CD10, HLA-DR, CD20, CD45RO, CD57, CD68 and κ/λ light chains of immunoglobulins.

Results

Histological evaluation

The 45 tissue specimens, obtained by extraction or apicectomy, were histologically diagnosed as shown in Table 2: 25 periradicular granulomas (55.55%), 17 periradicular cysts (37.77%) and 3 scar tissues (6.66%). Periradicular granulomas were characterized by the presence of granulomatous tissue infiltrated by inflammatory cells, whilst periradicular cysts consisted of a cavity lined with epithelium and surrounded by connective tissue. The presence of antibody producing plasma cells, as showed from the histological evaluation, was mainly moderate (++) and intense (+++). It was higher in cysts compared to granulomas, suggesting local humoral immune reactions in both the lesions (Table 2).

Immunohistochemical evaluation

The T_4/T_8 cell ratio is known to be an indicator of the host immunoregulatory status and T_4 helper cells were found in higher numbers than T_8 suppressor/cytotoxic cells. This ratio ranged approximately from 1 to 3 and greater (Table 3). Topographically, T_4 cells were identified more frequently in the connective tissue as a diffuse infiltration, whilst T_8 cells were more often found subjacent or within the epithelium.

The anti-CD10 MA, which reacts with germinal centre cells of lymphoid follicles and cells of acute lymphoblastic leukaemia, as well as mesenchymal cells, revealed few fibroblasts in two cases.

HLADR+ cells were only weakly observed in most of the samples (Table 4). These cells were found to be associated with the epithelium (Fig. 1), suggesting antigen-processing activity. Therefore, there is an indication that these cells, in combination with CD8 cells infiltrating the epithelium, could be related to epithelial cell proliferation.

Table 3 Percentage of the T_4/T_8 ratio in the tissues examined

CD4/CD8	1/1	3/2	2/1	3/1	>3/1	>>3/1	Not evaluated
%	9	9	16	4	47	13	2

Cell counts (%)	HLA-DR$^+$	CD3	CD10	CD20	CD45RO	CD68	CD57
–	20	2	93	18	7	24	78
+	62	85	5	60	60	36	22
++	11	11		18	29	22	
+++	7			4	4	18	
++++							
Not evaluated		2	2				

Table 4 Percentage of HLA-DR+ cells, CALLA cells, B and T lymphocytes, macrophages and NK cells infiltrating the tissues examined

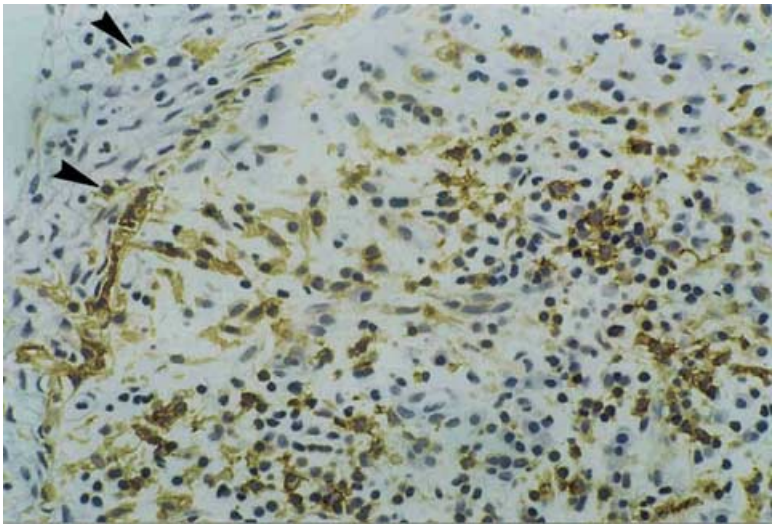

Figure 1 Immunohistochemical staining for HLA-DR positive cells. HLA-DR expression of antigen-presenting cells associated with the epithelium (arrows) and in the connective tissue (paraffin section; anti-HLA-DR ABC stain; ×400).

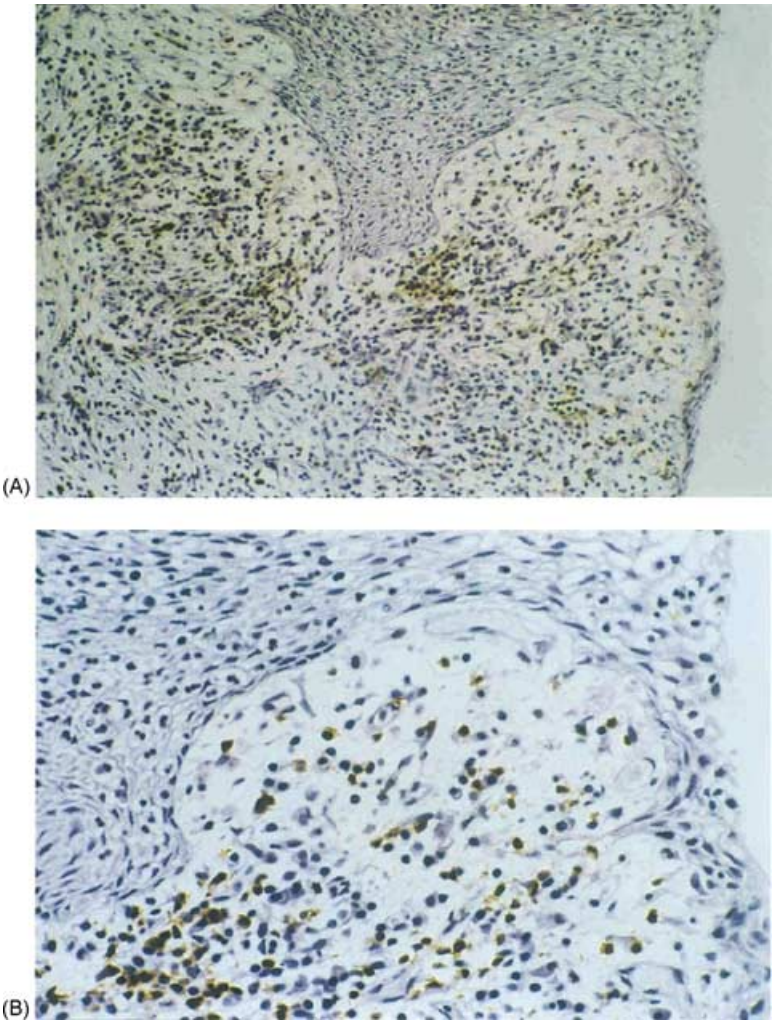

Figure 2 Immunohistochemical staining for T lymphocytes. CD45RO expression of lymphocytes in the periradicular tissue (paraffin section; anti-Pan-T ABC stain). (A) Original magnification ×200; (B) original magnification ×400. Numerous Pan-T-stained cells infiltrating the connective tissue under the epithelium layer.

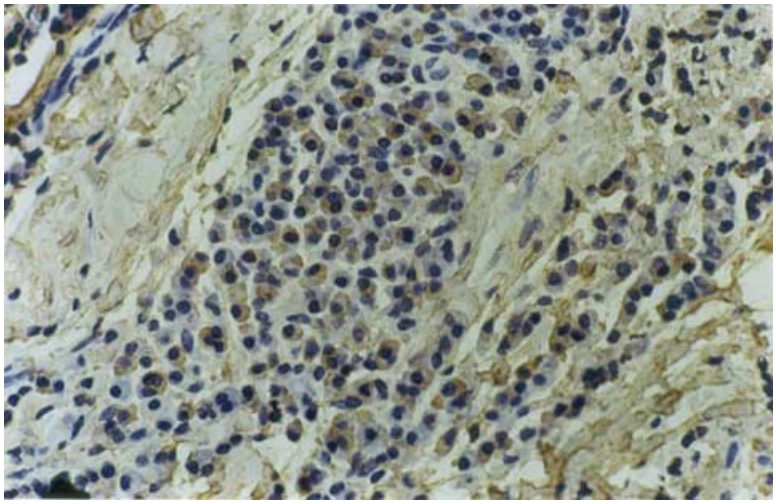

Figure 3 Immunohistochemical staining for κ light chains of immunoglobulins in the periradicular tissue (paraffin section; antikappa ABC stain; ×400).

Lymphocytes were also numerous in the inflammatory infiltrates. When applied, anti-CD3 MA showed a sufficient presence of T lymphocytes. T memory cells, as demonstrated by means of the anti-CD45RO MA (Fig. 2A,B), were present in most specimens in weak (+) and moderate (++) levels (Table 4). The number of T lymphocytes slightly exceeded that of B lymphocytes as shown by the anti-CD20 MA, whilst plasma cells, the B-cell descendants, were regularly observed as previously reported, indicating that the majority of the lesions were in a developing stage. Furthermore, the presence of plasma cells was confirmed by the quantitative assessment of κ and λ light chains of immunoglobulins (Fig. 3), which were often present in ratios between 3/2 and 3/1 (Table 5).

Table 5 Percentage of κ/λ ratio of light chains of immunoglobulins in the tissues examined

κ/λ	–	1/1	3/2	2/1	3/1	>3/1	Not evaluated
%	4	4	33	25	18	9	7

By applying anti-CD57 and anti-CD68 MA, natural killer (NK) cells and macrophages were detected. Only a few scattered NK cells were demonstrated in few cases, whilst macrophages (Fig. 4) were found in weak (+), moderate (++) and intense (+++) numbers (Table 4).

The mean values of cell populations and variables were compared amongst the two different histological types of periradicular lesions (periradicular granulomas

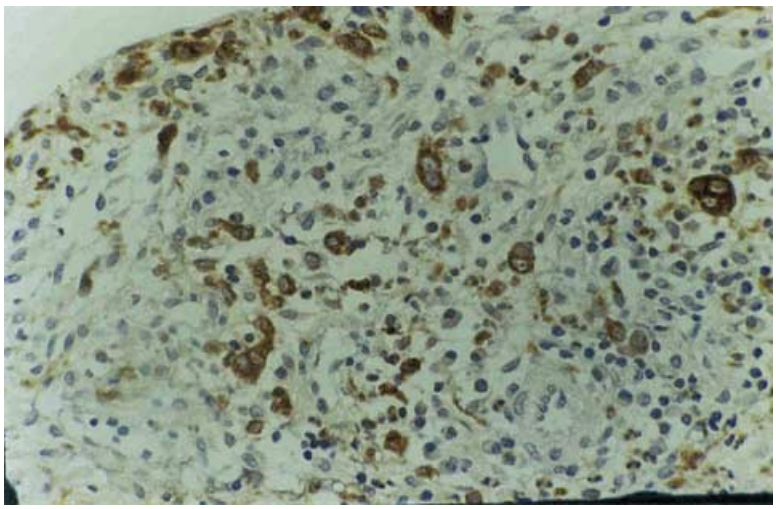

Figure 4 Immunohistochemical staining for macrophages. A diffuse infiltration of cells expressing CD68 antigen in the periradicular tissue. (paraffin section; anti-CD68 ABC stain; ×400).

Table 6 Differences of inflammatory infiltrate cells and variables in periradicular granulomas and cysts

	P-values	
Depended variables	Fisher's exact test	Wilcoxon test
CD4/CD8	0.337	0.57
CD3	0.62	0.42
CD10	1.00	–
HLA-DR	0.95	0.63
CD20	0.95	0.88
CD45RO	0.80	0.96
CD57	0.162	–
CD68	0.22	0.1025
κ/λ	0.67	0.77

and cysts). No statistically significant differences were found between the two groups ($P > 0.05$) for any of the variables or cell counts appearing in Table 6.

Discussion

This study was designed to determine the cellular profile of human chronic periradicular lesions using immunohistochemical methods in order to study the differences in the cell infiltrate of periradicular granulomas and cysts.

All biopsies were selected from random and representative sites, and complete clinical data were collected. The majority of the specimens were derived from asymptomatic and chronic periradicular lesions.

Histological examination of the haematoxylin and eosin-stained sections showed 25 periradicular granulomas, 17 periradicular cysts and 3 scars, according to the criteria for classification of periradicular lesions (Nair 1997). The proportion of granulomas to cysts was similar to previous observations (Johannessen *et al.* 1983, Tani *et al.* 1992) and most of the lesions exhibited features of localized, chronic inflammation. The hallmarks of chronic inflammation indicate a persistent inflammatory response induced by prolonged exposure of the periradicular tissues to various agents evoking an immunological reaction (Piattelli *et al.* 1991).

The immune cells infiltrating periradicular tissues, as well as their messenger molecules, e.g. immunoglobulins, were observed in both periradicular granulomas and cysts by immunohistochemistry.

The immunoenzymatic techniques, APAAP and ABC, are highly valid and sensitive methods and use a panel of specific monoclonal antibodies (Kopp & Schwarting 1989, Piatteli *et al.* 1991, Marton & Kiss 1993, Tani *et al.* 1992, 1996).

The proportions of immune cells and molecules were compared within periradicular granulomas and cysts and no significant differences were found between these lesions. This result is consistent with previous reports (Torabinejad & Kettering 1985, Gao *et al.* 1988a, Matsuo *et al.* 1992).

T and B lymphocytes and macrophages were found to comprise the majority of the inflammatory infiltrate. These cells have been reported (Nilsen *et al.* 1984, Torabinejad & Kettering 1985) to play essential roles in cell-mediated mechanisms involved in chronic inflammation. On the other hand, NK cells were rarely found. NK cells are thought to play an important role in tissue damage as well as in modulation of B-cell activity (Kimata *et al.* 1987).

Examination of the present material showed that T and B lymphocytes were equally distributed in the tissues. T-cell population was slightly higher than B cells. This respective population is in agreement with findings of Lukic *et al.* (1990), who showed greater number of T cells than B cells, when compared in the same specimens. Immunohistochemical analysis of different subsets of periradicular T cells revealed the host immunoregulatory status. It was detected that T helper/inducer cells were predominant in the lesions and the T_4/T_8 ratio, ranged approximately from 1 to 3 and greater, being consistent with the inflammation of periradicular tissues. This finding was in agreement with previous reports (Kopp & Schwarting 1989, Tani *et al.* 1992, Marton & Kiss 1993) on this ratio. One of the limitations of this study was that simple evaluation of phenotypic markers of T-cell subsets does not adequately reflect the immune processes that may occur (Takahashi 1998). Therefore, the effector functions of these cells' activity should be assessed.

The presence of macrophages in tissues (Torabinejad & Kettering 1985, Kopp & Schwarting 1989) confirms the major role of these cells in the pathogenesis of lesions. Both B and T lymphocytes respond to antigens, after processing and presentation by macrophage surface structures, encoded by the HLA-DR system (Kopp & Schwarting 1989). Also, when activated, macrophages may participate in triggering lesion expansion (Suzuki *et al.* 1999).

Genetically, HLA-DR-controlled macrophages may be essential in regulating almost all steps of inflammatory and immunologic processes (Meikle *et al.* 1986). In this study, the HLA-DR positive cells, including macrophages, dendritic cells and activated B cells, were detected along the epithelium. This observation indicates that in most of these specimens the antigen-presenting cells and the

T suppressor/cytotoxic cells were associated with newly formed as well as pre-existing epithelium. Kopp & Schwarting (1989) considered that the HLA-DR restriction, in connection with macrophages, in part modulates the T-cell differentiation. They also speculate that the T-cell-dependent reaction can be intensified or maintained by epithelial components. According to Gao *et al.* (1988b), during cyst formation, proliferating epithelium is assocciated with a local accumulation of various types of immune cells and is therefore stimulated by the soluble mediators of inflammation like cytokines, and influencing epithelial phenotype. Furthermore, Cury *et al.* (1998) reported that any change in epithelium status could indicate the biological activity of periradicular lesions.

Observations of this study also suggested humoral immune reactions in the tissues examined. Within the inflammatory infiltrate, immunoglobulin-containing cells were detected and the final differentiation of B cells to plasma cells was confirmed. Extrapolating the presence of these cells plus the molecules of humoral immunity (κ and λ light chains) in both lesions types, granulomas and cysts, a periradicular lesion should be considered prone to transformation. This speculation is providing the support that a conversion of a stable lesion to a progressive one histologically involves a shift from a T lymphocyte to a plasma cell dominance in the inflammatory cell infiltrate.

Conclusions

The results confirm that T and B lymphocytes and macrophages comprise the majority of the inflammatory infiltrate. This finding, combined with the presence of plasma cells and κ and λ light chains of immunoglobulins, indicates that both humoral and cell-mediated immune reactions are present in human periradicular lesions.

In periradicular granulomas, immunocompetent cells dominated. In periradicular cysts, the local immune reaction was correlated with epithelium, reinforced by macrophages, whilst the presence of HLA-DR+ cells elucidated the genetic control of infection-induced immune response.

The differences in the proportions of immunocompetent cells, when detected between periradicular granulomas and cysts, were not significant, indicating a similar pathogenesis of these lesions. Both lesion types, granulomas and cysts, are part of two different developing stages of the chronic periradicular pathology due to the process of immune reactions that cannot be inhibited.

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