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Histological changes in the tonsils of patients infected by Epstein –barr virus

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Summary

This study was conducted on 50 subjects whom had visited the AL-Sadder Medical City and Al-Hakeem hospital in AL- Najaf governorate. The subjects of this study were chronic or recurrent tonsillitis patients, their age ranged from 4 to 33 years.. The biopsy of tonsils were fixed in 10% neutral buffered formalin and then processed in routine histopathological techniques.

The histopathological changes revealed infiltration of the inflammatory cells, multinucleated giant cell, germinal hyperplasia and large abnormal lymphocytes can be seen. Slight-moderate lymphocyte infiltration and/or diffuse lymphocyte infiltration leading to the defect in the surface epithelium of tonsils.

Introduction

Tonsillitis is the inflammation of the tonsils as a result of infection mostly caused by a virus especially the Epstein-Barr virus Adenoviruses, influenza virus, para influenza virus and Enteroviruses and is often preceded by a cold (a runny nose, cough and sore throat). Fewer cases (about one in seven) are caused by bacteria (Hutt-Fletcher, 2007).

Primary Epstein-Barr virus (EBV) infection in childhood is typically asymptomatic, but infection later in life results in a mononucleosis with the illness severity ranging from asymptomatic to requiring hospitalisation. The illness is generally short-lived (four to six weeks following onset of symptoms), but persistent disabling symptoms (lasting up to 6 months or longer) are well described in around ten percent of individuals(Turk *et al*., 2006).

Epstein-Barr virus (EBV) is a common human pathogen, affecting 80% of adults in the US. Since its discovery in 1964, EBV has been etiologically implicated in an increasing number of human diseases, such as infectious mononucleosis (IM) (Baltz, 2002). EBV has also been associated with B cell lymphomas in immunosuppressed individuals, including both transplant patients and patients with AIDS. EBV is classified as a member of the herpesvirus family based upon its morphological characteristics (Schooley, 2001). Although primary infection with EBV during childhood is usually asymptomatic, nearly one-half to two-thirds of primary infections with the virus in older adolescents and young adults result in overt clinical disease such as IM (Baltz, 2002). IM is an acute, self-limited lymphoproliferative disease caused by EBV. When primary infection is delayed until young adulthood and adolescence, however, there is about a 50% chance that it will occur with the classic clinical manifestations associated with IM. The virus is transmitted via saliva and primary infection in the childhood, as it is common in most parts of the world, remains asymptotic in most cases. In industrialised countries primary infection is frequently delayed into young adulthood or adolescence and then may cause IM with fever, lymphadenopathy, sore throat, and fatigue, usually resolving within a few weeks (Balfour *et al*., 2005). During IM EBV-infected B-lymphoid blasts are activated and proliferate in the paracortex of tonsillar lymphoid tissue. During IM the proliferating EBV-infected B-cells induce a cytotoxic T-cell response against latent and lytic viral genes (Young and Murray, 2003; Pudney *et al*., 2005). Shedding of EBV from the oropharynx occurs intermittently in EBV-seropositive individuals. During such periods of EBV shedding other persons may become infected. When EBV enters the oropharyngeal cavity ,it penetrates local lymphoid tissue

(lingual-, palatine-, and pharyngeal tonsils) (Fafi-Kremer *et al.*, 2005). Squamous epithelium covering the lymphoid tissue dips into the tonsillar crypts where B-cells are densely situated. Following primary infection, these B cells are the first to be infected**.**

2-Methods and materials

2-1 Subjects

Fifty patients complained from chronic or recurrent tonsillitis had ttained AL-Sadder medical city and Al-Hakeem hospital in AL-Najaf city they their ages ranged from 4-33 years old. Patients with chronic tonsillitis complained of sore throat. The physical examination showed exudative tonsillitis accompanied by exudative and/or erosin of the oropharyngeal mucosa and cervical lymphadenopathy,

2.2. Tonsils

Human palatine tonsils samples were collected from routine tonsillectomies performed at AL-Sadder medical city and Al-Hakeem hospital in AL- Najaf city, the tonsils were fixed in 10% buffered formalin until processing.

2-3. Tissue processing

Tissue processing was concerned with the diffusion of various substances into and out of stabilizes porous tissues. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside blocks of tissue. Tissue specimens received in the pathology laboratory have a request form that lists the patient information and history along with a description of the site of origin. The specimens were accessioned by giving them a number that will identify each specimen for each patient.

3.2.2.1. Preparation of tissue sections

Bancroft and Stevens (1999) had prepared tissue sections and by the way, which included the following steps:

1- Dehydration:

Passed in the concentrations of progressive forms of ethanol (70, 80, 90, and 95,100%). For the period (1.5 - 2) hours in each concentration in order to remove water.

2- Clearing:

Samples were cleared with xylene, twice for a period of (1.5-2) hours for each time in order to remove the clearing solution from the tissue.

3- Infiltration:

Infiltrate the samples with molted paraffin wax (56-58 C˚) by placing the samples twice (1.5-2) hours each time.

4- Embedding:

Buried samples in a container with specific templates to molten paraffin wax and left to harden.

5- Sectioning:

Tissues were sectioned into (2-4µm) thickness using a rotary microtom and fixed models on the slides using the adhesive (Meyers albumin) and then put the slide in the oven at a temperature of (56- 58c˚) for (20) minutes to remove excess wax.

6- Staining

Histological Staining – Haematoxylin & Eosin (H&E)

The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue. The routine stain is that of hematoxylin and

3.2.3. Microscopic examinations

The stained section on the slide was examined by using light microscope (Olympus, Japan). Histopathological changes were reported by pathologist.

4- Result and disscution

The result of microtom sections from tonsillar biopsy showed, infiltration of the inflamed tonsils. Also a through looking the stained section with H & E of the tonsils, many multinucleated giant cell can be seen, germinal hyperplasia and large, abnormal, lymphocytes (Figures 1, 2, 3).

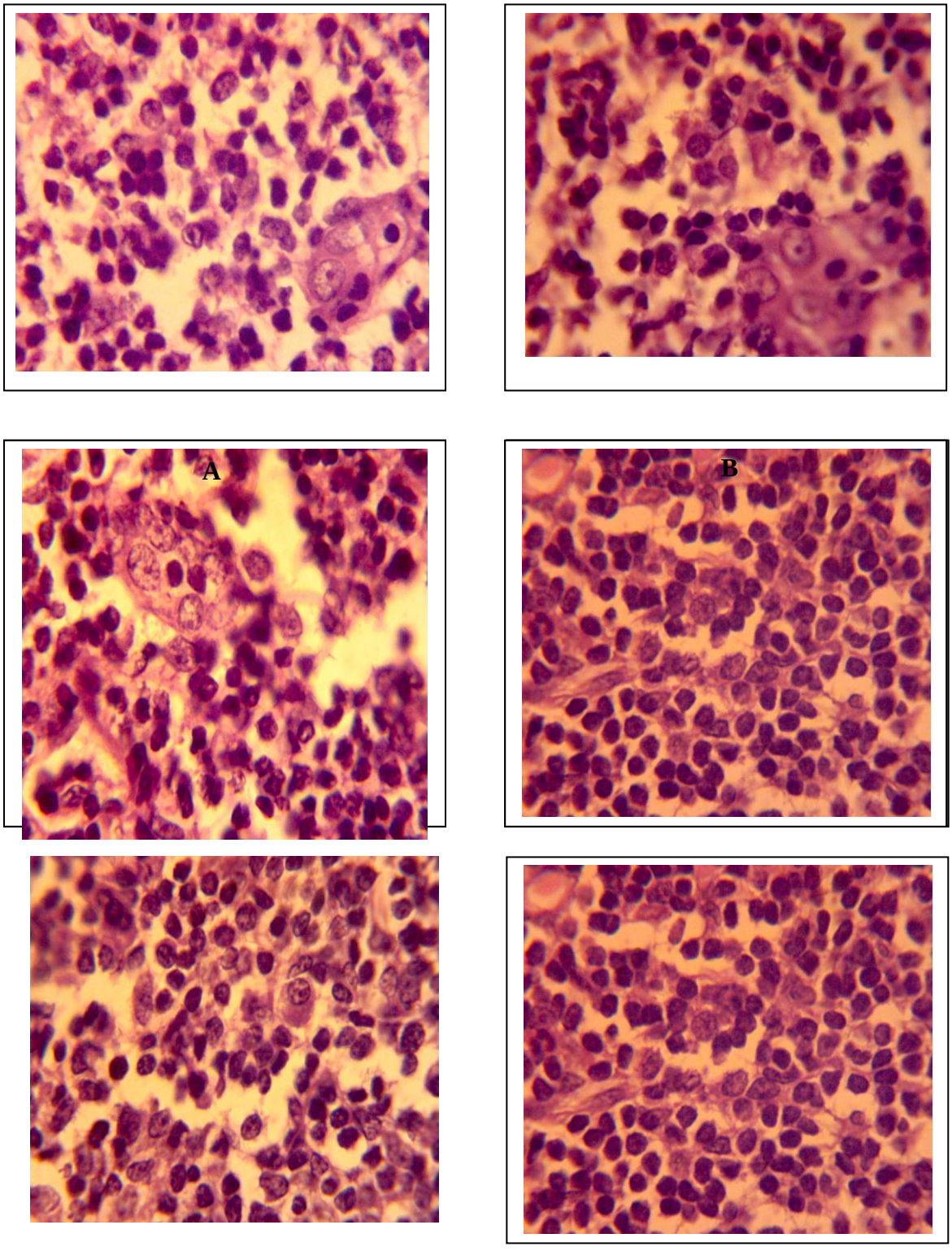

Figure 1: Diffuse lymphocyte infiltration leading to the defect in the surface epithelium (Hematoxylin-eosin stain, original magnification X40).

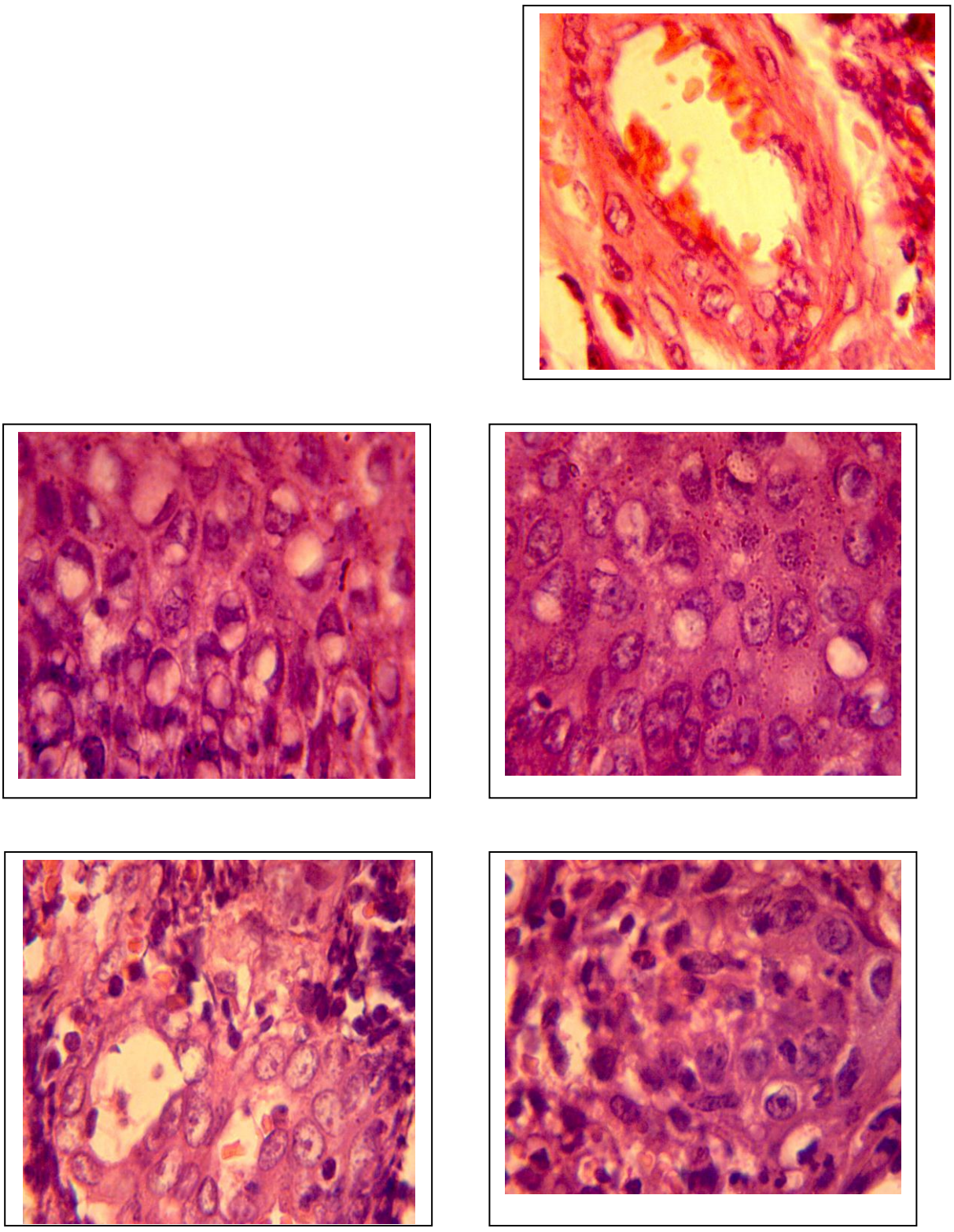

Figure 2: The presence within the surface epithelium of scattered small lymphocyte groups in moderate lymphocyte infiltration in the surface epithelium region (Hematoxylin-eosin stain, original magnification, X 40).

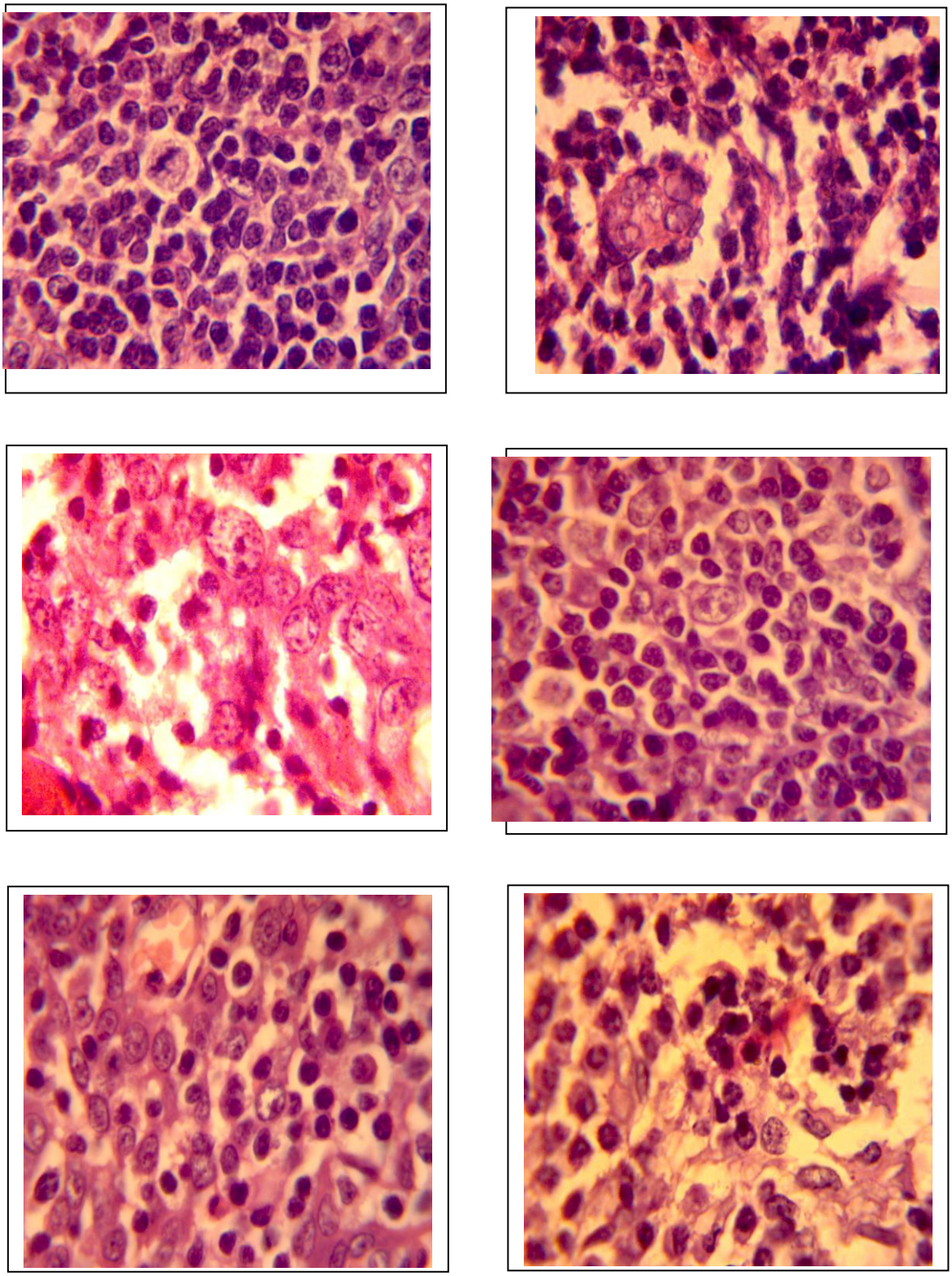

Figure 3. The presence of giant cell in tonsils (Hematoxylin-eosin stain, original magnification, X 40)

In microtom sections from tonsillar biopsy, infiltrations of the inflamed tonsils were heavily infiltrated with lymphocytes and neutrophils as they represent the primary defense mechanism. Also through looking the stained section of the tonsils, many multinucleated giant cells can be seen, they are pathognomonic for EBV and /or CMV infection. Slight-moderate lymphocyte infiltration and/or diffuse lymphocyte infiltration leading to the defect in the surface epithelium not present in normal tonsils. Therefore, combinations of three findings are fairly diagnostic for chronic tonsillitis.

Windfuhr *et al.*, (2005) reported that tissue from tonsils exhibit germinal hyperplasia , large, abnormal and infiltrated of lymphocytes, non neoplastic T lymphocytes aberrant lymphocytes are basophilic with a vacuolated cytoplasm. EBV induces a proliferation of B lymphocytes in the lymphoid tissue. When EBV infects B lymphocytes, they become transformed. Histologically, these transformed lymphocytes are large and atypical, and they frequently have prominent nucleoli. The inset is a higher magnification of the EBV-infected B lymphocytes. The tonsils in the patient in the case would look like this during the chronic infection.

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