Increased Numbers of Langerhans Cell and Expression of HLA-Dr Antigen in the Giant Papilla of Patients With Giant Papillary Conjunctivitis

Tae-Hoon Choi, M.D., Myung-Kyoo Ko, M.D.,* and Joon-Kiu Choe, M.D.*

Department of Ophthalmology, College of Medicine, Hallym University, *Department of Ophthalmology, College of Medicine, Hanyang University, Seoul, Korea

A study of histopathologic changes, ultrastructure, and expression of the HLA-Dr antigen within the giant papillae of patients with giant papillary conjunctivitis was performed to determine whether cell-mediated immune response is related to this condition. Conjunctival giant papillae from ten patients with giant papillary conjunctivitis were examined by light and electron microscopy and by the indirect immunofluorescent staining method with HLA-Dr antibody. The infiltration of eosinophilic neutrophils and granules was most prominent, with the occasional infiltration of mast cells, as shown by light microscopy. The infiltration of activated fibroblasts and Langerhans cells was also observed. Cells expressing HLA-Dr antigen were also markedly increased, as shown by the immunofluorescent method. These findings suggest that delayed hypersensitivity may, along with the processes of antigen presentation by HLA-Dr-expressing (including Langerhans) cells, contribute to the pathogenesis of giant papillary conjunctivitis.

Key words: Birbeck granule, HLA-Dr, Langerhans cell, giant papillary conjunctivitis

INTRODUCTION

Giant papillary conjunctivitis is known to occur in contact lens wearers and in cases of suture exposure following prosthetic insertion or ocular surgery.1-8 Contact lens wearer with no previous symptoms develop a mucous discharge, mild itching sensation, intolerance to the lens and giant papillae in the upper palpebral conjunctiva.3-7 Histologically, the epithelium and stroma of conjunctival papilla are characterized by mainly mast cells and eosinophils, the infiltration of basophils and plasma cells, a cluster of monocytes and fibroblasts, newly-formed collagens and an increased number of lymphocytes and polymorphonuclear leukocytes.1,3,4,9,13 It is known that increased tear levels of IgE and IgG, and occasionally IgM, the presence of plasma cells containing IgE and a decreased concentration of lactoferrin are frequent findings in patients with giant papillary conjunctivitis.13-18

Though both the immediate type of hypersensitivity reaction and cell-mediated immune processes are known to be associated with the pathophysiology of giant papillae,20-23 this immediate hypersensitivity is most frequently involved.1,3,15 We performed this study, observing expression of HLA-Dr antigen and Langerhans cells within the giant papillae, to determine whether cell-mediated immune response is related to giant papillary conjunctivitis.
MATERIALS AND METHODS

Excision of giant papillae on the upper palpebral conjunctiva of patients with giant papillary conjunctivitis was performed. To examine by light microscopy the infiltration of cells, the tissue was stained by hematoxylin-eosin; to observe Langerhans cells it was prepared for electron microscopic examination, and to observe any increase of cells expressing HLA-DR antigen it was prepared for indirect immunofluorescent staining using this antigen.

Patients with vernal conjunctivitis were excluded from the study. Those with giant papillary conjunctivitis ranged in age from 11 to 63; seven eyes had worn soft contact lens and three eyes involved suture exposure after a cataract operation. All ten specimens were stained by hematoxylin-eosin; five were examined by transmission electron microscopy and the other five by the indirect immunofluorescent staining method using HLA-DR antibody. For this staining, tissues were embedded in OCT (Mile, U.S.A.), frozen to -70°C, and cut into thin sections using cryomicrotome. Each section was stained by mouse HLA-DR antibody (Biogenex, U.S.A.) and then by FITC-conjugated goat antimouse antibody (Vector, U.S.A.). Each specimen was examined by fluorescence microscope to observe cells expressing HLA-DR antigen. As a control, five conjunctival tissues obtained during pterygium excision were stained using the same method.

Electron microscopic examination was performed for ultrastructural analysis. Specimens were fixed in 2% glutaraldehyde and postfixed in osmium tetroxide. Each sample was dehydrated in ethylalcohol and then embedded in Epon. An 1 μm-thick section was made by microtome and stained with toluidine blue. After the area of examination was determined by light microscopy, ultrathin sections were made. They were double stained by uranyl acetate and lead citrate and then examined by transmission electron microscopy (Hitachi-600, Japan).

RESULTS

On light microscopic examination, there were infiltrations of inflammatory cells, especially scattered eosinophils and eosinophilic granules. Spindle-shaped fibroblasts were prominently infiltrated along the vessels, but on rare occasions there was infiltration of basophils, mast cells, and plasma cells (Fig. 1).

On indirect immunofluorescent staining with HLA-DR antibody, dendritic cells appeared in the conjunctival epithelium and stroma of control eyes (Fig. 2), but dendritic or other pleomorphically positive cells in the conjunctival stroma of giant

Fig. 1. Infiltration of inflammatory cells such as eosinophils, basophils and plasma cells in the papillae of giant papillary conjunctivitis (hematoxylin-eosin, ×400).

Fig. 2. Cells expressing HLA-DR antigen on basal epithelium and stroma of control conjunctiva (immunofluorescent stain, ×400).
papilla were more prominent than those of control eyes (Fig. 3). Dendritic cells expressing HLA-DR antigen were regarded as Langerhans cells. Electron microscopic observation showed that fibroblasts near the vessels of the stroma had prominent nucleoli with indented nuclear membrane and cytoplasm with numerous rough endoplasmic reticula. There was infiltration of inflammatory cells such as eosinophils with electron-dense granules, plasma cells, mast cells, and monocytes (Figs. 4, 5). Mucous plaque within the goblet cells of surface epithelium, as well as mast cells, were found in the stroma of conjunctival papillae. Langerhans cells were found in five cases of conjunctival giant papillae and showed rough endoplasmic reticula and numerous tennis racket-shaped Birbeck granules within the cytoplasm (Figs. 6, 7).

**DISCUSSION**

Light microscopy showed infiltration of eosinophils and their granules, and of basophils, plasma cells, and polymorphonuclear leukocytes. On electron microscopic examination, there was infiltration of eosinophils, basophils, plasma cells and mast cells, and this is compatible with other findings concerning histopathologic characteristics of giant papillary conjunctivitis.\(^4,9\) Besides these

![Fig. 3](image-url) **Fig. 3.** The increased numbers of dendritic cells expressing HLA-DR antigen on the conjunctival epithelium and stroma of conjunctival papillae (immunofluorescent stain, \(\times\) 400).

![Fig. 4](image-url) **Fig. 4.** Infiltration of inflammatory cells such as activated fibroblasts (arrows), eosinophils (arrowheads), and lymphocytes in the stroma of conjunctival papillae (\(\times\) 3,500).

![Fig. 5](image-url) **Fig. 5.** Infiltration of inflammatory cells such as plasma cells (arrowheads), lymphocytes, activated fibroblasts (arrows) within the stroma of conjunctival papillae (\(\times\) 4,000).

![Fig. 6](image-url) **Fig. 6.** Langerhans cells with numerous tennis racket-like rods (arrowheads), mitochondria and Golgi bodies within cytoplasm of the basal epithelium of conjunctival papillae (\(\times\) 8,000).
findings, there were numerous rough endoplasmic reticula, prominent nucleoli, and indented nuclear membranes in the cytoplasm of fibroblasts, which showed increased activity. This suggests a severe inflammatory response and these cells are thought to secrete various extracellular matrices, especially collagen, which are major components of extracellular matrix in conjunctival papillae.

Indirect immunofluorescent staining using HLA-DR antibody showed a marked increase of cells expressing HLA-DR antigen compared to controls; these cells were dendritic Langerhans cells as well as round or ovoid cells which were considered to be macrophages. Typical Langerhans cells containing Birbeck granules were observed with electron microscopy. Langerhans cells have various cytological characteristics including an indented nuclear membrane, a lack of desmosome or tonofilaments, a well developed Golgi apparatus and Birbeck granules with a distinctive intracellular structure.2,4

Birbeck granules reveal various features according to different electron microscopic cutting positions, but on occasion it resemble a tennis racket because 3-dimensionally, a granule is composed of a rod with a round vesicle at its end. Langerhans cells are known to be present in normal conjunctiva and corneal limbus.25-30 Gillette et al.25 reported that the numbers of these cells decrease in the conjunctiva, and limbal, peripheral and central cornea. They are also associated with contact hypersensitivity and with allograft rejection in conjunction with conjunctiva-associated lymphoid tissue following penetrating keratoplasty.31

Gillette et al.25 supposed that the increased cellularity of Langerhans cells in corneal limbus is related to the infiltration of inflammatory cells and development of ulcers in this area. As on HLA-Dr antigen, glycoprotein on the surface of a Langerhans cell plays an important role in expressing the immune system, especially the interaction between a macrophage and a T-lymphocyte, recognizing Ag combined with a macrophage by a T-lymphocyte.26,29,30

Bhan et al.20 observed increased helper T-cells in vernal keratoconjunctivitis or ocular pemphigoid, cytotoxic T-cells in graft-versus-host disease and an increased number of Langerhans cells in certain circumstances of pathologic conjunctiva. Langerhans cells act as an antigen presenter in IgA immune reaction and as a T-cell mediated immune response on the surface of ocular tissue. They are more effective than macrophages in combining with surface Ag and inducing cell-mediated immune response. These cells process the foreign antigen and then migrate to the regional lymphoid tissue, probably via lymphatic channels. Langerhans cells initiate T-cell sensitization against sensitizing substances such as haptons, water-soluble protein in the skin, and this ability to express antigenicity plays an important role in the pathogenesis of contact dermatitis. The ability to express antigenicity of ocular surface therefore depends on Langerhans cells and CALT (conjunctiva associated lymphoid tissue).25

In giant papillary conjunctivitis the most prominent findings are infiltration of eosinophils and mast cells; they are findings which correspond to the immediate hypersensitivity reaction, a well-known histopathologic feature of giant papillae caused by vasoactive amines secreted from mast cells or basophils and other mediators released from inflammatory cells infiltrated in conjunctiva in response to persistent irritation by suture material, and the consequent proliferation of fibrovascular cells.6,15

The existence of increased numbers of cells expressing HLA-Dr antigen, together with the clinical finding that giant papillae occur several
weeks to months after contact lens wear or postoperative suture exposure implies that the pathogenesis of giant papillae is, besides the immediate hypersensitivity reaction, closely related to the delayed hypersensitivity response caused by the release of cytokines from T-lymphocytes which recognize antigens processed by cells expressing HLA-Dr antigen.

REFERENCES


