Localization of intercellular adhesion molecule-1 in middle ear cholesteatoma

H. Shinoda · C. C. Huang

Abstract Intercellular adhesion molecule-1 (ICAM-1) may have a role in acquired cholesteatoma, which is usually associated with an inflammatory reaction occurring in the middle ear cavity. The presence of ICAM-1 in human cholesteatomas was demonstrated by an immunoblotting assay using a specific monoclonal anti-ICAM-1 antibody after protein extraction. Distribution of ICAM-1 in the cholesteatoma tissues was then studied by avidin-biotin-peroxidase complex staining. ICAM-1 appeared to be localized on keratinocytes in all layers of the epithelium and on Langerhans cells in both the epithelium and granulation tissue of cholesteatoma. ICAM-1 was not found in the epidermis of normal external ear canal skin, normal tympanic membrane or normal facial skin, but significant staining was seen on keratinocytes of hair follicles and glands in the facial skin. The present study is the first to demonstrate ICAM-1 in cholesteatoma and suggests that it may have an important role in the clinical development of cholesteatoma, including migration, adhesion and proliferation of lymphocytes, Langerhans cells and keratinocytes.

Key words Cholesteatoma · Intercellular adhesion molecule · Interleukin-1 · Tumor necrosis factor-alpha

Introduction

Intercellular adhesion molecule-1 (ICAM-1) is a glycoprotein that consists of five immunoglobulin-like domains, a transmembrane region and a short cytoplasmic tail. It has a molecular weight of 90–114 kDa, with a protein core of about 55 kDa [22]. ICAM-1 is a member of the immunoglobulin supergene family [18, 20] and is known to play a role in mediating cell-to-cell adhesion during inflammatory responses and transendothelial migration of leukocytes [6]. ICAM-1 has also been found to demonstrate a pivotal role in cell-to-cell interaction in the immune system [4, 5]. Various cell types can express ICAM-1, including vascular endothelial cells, lymphocytes, fibroblasts, tissue macrophages and keratinocytes, although very little or no ICAM-1 is detectable on the cell surface under normal conditions. In vitro, ICAM-1 can be induced by such inflammatory mediators as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [6, 27]. TNF-α and IFN-γ in particular have been shown to induce keratinocytes to produce ICAM-1 [9]. ICAM-1 acts as a ligand for lymphocyte function-associated antigen-1 (LFA-1), and its expression has been correlated with infiltration of lymphocytes into inflammatory sites [17, 21].

Acquired middle ear cholesteatoma is characterized by the accumulation of keratinizing epithelium resulting from the hyperproliferation and differentiation of epithelial cells. Although the pathogenesis of acquired cholesteatoma is still not completely understood, its development is known to be associated with a chronic inflammatory process [11]. Since ICAM-1 has been found to have a significant role in many inflammatory diseases, we wanted to determine whether it was also involved in the development of cholesteatoma. In order to delineate a possible role, we first examined the presence and distribution of ICAM-1 in cholesteatoma epithelium in the present study.

Materials and methods

Tissue specimens

Specimens were obtained from patients during middle ear or tympanomastoid operations. Protocols for using specimens for this study were approved by the Columbia University Institutional Human Use Committee Review Board and all studies were in compliance with United States federal, state and local regulations concerning the use of human subjects and materials in research. Specimens included 12 acquired cholesteatomas, 3 normal external ear canal skins and 2 normal tympanic membranes. Two specimens of normal human facial skin also were obtained from patients under-
going elective facial plastic surgery. The ages of the patients with middle ear cholesteatomas ranged from 21 to 56 years, with a median of 42 years. Following the surgical removal, all specimens were fixed in 10% phosphate-buffered formalin, dehydrated, embedded in paraffin, and sectioned in 6 μm thicknesses. These sections were then used for immunocytochemical studies.

Gel electrophoresis and immunoblotting

Four specimens of cholesteatoma tissues were homogenized in a glass homogenizer and sonicated three times for 5 s at 4°C in 50 mM TRIS-HCl, pH 7.6, containing 100 μg/ml phenylmethylsulfonyl fluoride, a protease inhibitor. Tissue homogenates were then centrifuged at 3,000 g for 5 min at 4°C and supernatants collected for immunoblotting. Protein extracts (80 mg protein/well) were subjected to electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide slab gel (SDS-PAGE) according to the procedure described by Laemmli [15]. All proteins separated on the gel were transferred electrophotographically onto a nitrocellulose membrane.

ICAM-1 in the nitrocellulose membranes was identified by immunoblot analysis in the following steps. Nitrocellulose membrane strips were first incubated with 0.2% glutaraldehyde for 30 min at room temperature, followed by incubation with 3% non-fat milk and 1% normal horse serum (Oncogene Science, Uniondale, N.Y.) for 30 min to block the non-specific binding sites. Strips were then incubated with 5 μg/ml monoclonal mouse anti-ICAM-1 antibody (CD54, Amac, Westbrook, Me.) at 4°C overnight followed by biotin-conjugated horse anti-mouse IgG (Oncogene Science) for 30 min at room temperature. Strips were next incubated with pre-mixed avidin and biotin-peroxidase (Oncogene Science) for 30 min. Finally, ICAM-1 was identified by a blue color band as assessed with TMB membrane peroxidase substrate (Kirkegaard & Perry, Gaithersburg, Md.) for 30 s at room temperature. For control staining, strips were incubated with normal mouse IgG to replace primary antibody.

Immunocytochemical staining

For immunolocalization of ICAM-1 and LFA-1, cholesteatoma tissue sections were stained with avidin-biotin-peroxidase complex. In brief, the procedure comprised the following incubation steps: (1) 1.0% hydrogen peroxide for 30 min; (2) normal horse serum for 30 min; (3) monoclonal anti-ICAM-1 antibody or monoclonal anti-LFA-1 antibody (CD11a; Dako, Carpinteria, Calif.), 10 μg/ml for 90 min; (4) biotin-conjugated horse anti-mouse IgG for 30 min; (5) premixed avidin and biotin-peroxidase for 30 min; (6) 0.01% hydrogen peroxide–0.05% 3,3'-diaminobenzidine–HCl in phosphate-buffered saline (PBS) for 3 min. Between each step, tissue sections were washed three times with PBS, for 5 min each time. Control staining was performed by substituting normal mouse IgG instead of primary antibody (step 5). Sections were mounted with glycerol in PBS and observed under an Olympus microscope.

Langerhans cells in cholesteatoma tissues were detected by the indirect immunoperoxidase method using a polyclonal rabbit anti-S100 protein antibody (Sigma, St. Louis, Mo.). Immunoperoxidase staining included the following incubation steps: (1) 1.0% hydrogen peroxide for 30 min; (2) 3% normal goat serum for 30 min; (3) polyclonal rabbit anti-S100 protein antibody, diluted 1:200, for 2 h; (4) peroxidase-conjugated goat anti-rabbit IgG (Kirkegaard & Perry), 2 μg/ml, for 30 min; (5) 0.01% hydrogen peroxide–0.05% 3,3'-diaminobenzidine–HCl in PBS for 3 min. As a control, rabbit anti-S100 protein antibody was substituted by rabbit non-immunized IgG or antibody absorbed with an excess amount of S-100 protein (tenfold by weight).

Results

Immunoblot analysis

Immunoblotting of protein extracts of the four cholesteatoma tissues showed the presence of ICAM-1 in each, with a molecular weight range of 90–114 kDa (Fig. 1).

Immunocytochemical staining

The human middle ear cholesteatomas including subepithelial granulation tissues demonstrated a moderate inflammatory reaction characterized by infiltration of mononuclear cells such as lymphocytes. After staining with avidin-biotin-peroxidase complex, ICAM-1 was detected as an intercellular brownish precipitate in all layers of keratinocytes in the epithelia of the cholesteatoma studied (Fig. 2A). ICAM-1 was also localized in cells showing dendritic processes in cholesteatoma granulation tissue (Fig. 2B). In control studies using normal mouse IgG, no staining of ICAM-1 was seen in either granulation tissue or epithelium (Fig. 2C). Normal external ear canal skin and normal tympanic membrane (Fig. 3 A, B) were not stained significantly for ICAM-1. Normal human skin also showed no significant staining on the epidermis for ICAM-1. However, an intense immunoreactivity of ICAM-1 was seen on keratinocytes of the hair follicle area (Fig. 4 A) and glands (Fig. 4 B) of normal human skin.