Heat Shock Protein 72/73 was Expressed Ubiquitously on Follicular Dendritic Cells in Lymphoid Follicles

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The precise localization of the heat shock protein (HSP) 70 family on follicular dendritic cells (FDC) is not fully understood. The aim of the present study was to clarify the expression of both the inducible and constitutive 72/73kDa HSP 70 protein on FDC. The expression of heat shock protein (HSP) 72/73 on FDC in various lymphoid follicles was investigated. Immunohistochemically, HSP72/73 was detected on FDC in all the lymphoid tissues and showed extensive labeling of the light zone. Immunoelectron microscopy also confirmed that HSP72/73 was located not only diffusely in the cytoplasm but was also dotted in the nucleus of FDC. Although mantle zone lymphocytes and vascular walls in lymphoid follicles were faintly HSP72/73 positive, the germinal center lymphocytes were all negative. Finally, *in situ* hybridization demonstrated distinctive follicular expression of HSP70 mRNA. These data clearly indicate that HSP72/73 is ubiquitously expressed on FDC, although the significance of HSP 72/73 expression on FDC remains a mystery.

Key words heat shock protein, HSP70, lymphoid follicle, germinal center, follicular dendritic cell

INTRODUCTION

In response to environmental, physiological, pathological, and chemical stresses, cells synthesize a kind of protein, called heat shock proteins (HSP) or stress proteins, that serve to protect cells from stress by mediating protein folding and assembly. HSP are induced not only during stress but are also constitutively expressed at low levels under normal physiological conditions[1,2]. HSP are also known to act as molecular chaperones and to mediate various events from protein synthesis to degradation, as well as being involved with translocation through membranes, refolding after denaturation, and regulation of protein function[3,4]. The 70 kDa HSP family is composed of the 72 kDa inducible HSP70 and the 73 kDa as the constitutive HSP70 (HSP72/73), the 75 kDa mitochondrial HSP70 (GRP75), the 78 kDa endoplasmic reticulumHSP70 (BiP, GRP78), and the 72 and 74 kDa peptide-binding HSP70 (PBP72/73)[5,6]. Various reports have emphasized the significance of HSP70 expression in diseases (viral, bacterial or parasitic infections, or fever, inflammation, malignancy or autoimmunity)[7–13]. The chaperone function of HSP70 might indicate a role in antigen processing and presenting[14]. When foreign particles are taken up by antigen presenting cells (APC), protein antigens are proteolytically degraded. Binding of the resulting peptides to HSP70 might rescue them from total degradation and preserve them to be translocated through cell membranes for further antigen presentation[15]. The peptide binding domain of HSP70 is similar to the major histocompatibility complex (MHC).

Furthermore, antibodies to HSP70 inhibit antigen-presentation[16]. Secondary lymphoid follicles contain two types of dendritic cells, follicular dendritic cells (FDC) and germinal center dendritic cells[17,18]. FDC are one of antigen retaining cells within the lymphoid follicles (LF). FDC trap and retain antigens in the form of immune complexes on their cell surfaces for long periods by complement activation[19] and are closely associated with the clonal expansion, selection and differentiation ofB cells[18,20,21]. FDC also help to maintain follicular structure with their intricate, entangled and arborizing cytoplasmic processes[18,22].

Received : September 11, 2000
Revised : January 25, 2001
Accepted : January 26, 2001

Journal of Clinical and Experimental Hematopathology Vol. 41 No. 1

To our knowledge, there are no published papers confirming the precise expression of HSP70 family on FDC in various lymphoid tissues, although previous reports have suggested that there is a close relationship between the HSP70 family and APC[14–16]. The aim of the present study was to clarify the expression of both the inducible and constitutive 72/73kDa HSP70 proteins on FDC in secondary LF using immunohistochemistry, immunocytochemistry, immunoelectron microscopy, and in situ hybridization. We demonstrate here the ubiquitous expression of HSP72/73 on FDC.

MATERIALS AND METHODS

Specimens

Specimens with secondary LF were examined, these included palatine tonsils from 10 patients with chronic tonsillitis, cancer-free gastric mucosa from 6 patients with gastric cancer, terminal ileal mucosa from 4 patients with colon cancer, appendical mucosa from 8 patients with acute appendicitis and tubal pregnancy, spleens from 5 patients with idiopathic thrombocytopenic purpura, and neck lymph nodes from 9 patients with reactive lymphadenitis. Warthin's tumor (3 cases), chronic sialoadenitis (3 cases), Hashimoto's disease (5 cases), and Kimura's disease (3 cases) were also examined.

Tissue specimens were fixed with periodate-lysine–2% paraformaldehyde (PLP) at 4°Cfor 6 h, and rinsed with a graded sucrose solution in 0.01 M phosphate-buffered saline (PBS), pH 7.4. For immunohistochemistry and in situ hybridization (ISH), PLP-fixed tissue specimens were embedded in Tissue-Tek II optimal cutting temperature compound (Miles Inc., Elkhart, IN), frozen in acetone cooled with dry ice and stored at −80°C.

Antibodies

The primary antibodies used for immunochemical staining in this study were: anti-HSP72/73 antibody (W27, mouse IgG2a; Oncogene Science, Uniondale, NY, USA), anti-FDC antibody (R4/23, mouse IgM; DAKO, Glostrup, Denmark), anti-CD23 antibody (MHM6, mouse IgG1; DAKO), anti-CD20 antibody (L26, mouse IgG2aκ; DAKO), anti-CD45RO antibody (UCHL-1, mouse IgG2a; DAKO), anti-CD62L antibody (SK11, mouse IgG2a; Becton Dickinson, Mountain View, CA, USA), and anti-CDw75 antibody (LN-1, mouse IgM; Nichirei, Tokyo).

Isolation of FDC-associated clusters and lymphocytes from germinal centers (GCs)

PLP-fixed tonsillar tissues were cut into 200 m-thick slices using a microslicer (D KT-1000, Dosaka EM, Kyoto). The GC of the sections were enucleated under a stereoscope and digested with collagenase (class IV, 450 U/ml, Sigma Chemical Company, St Louis, MO, USA) in PBS at 37°Cfor 30 min under gentle, constant stirring. The cell suspension was washed with PBS, passed through a 100 m nylon cell strainer (Becton Dickinson) to remove any non-digested tissue debris, and mounted on silane-coated glass slides using a cytospin centrifuge (Shandon, Pittsburgh, PA, USA) at 1500 g for 5 min. These cytospin preparations were used for immuno-cytochemical double staining to identify the presence of HSP72/73 on FDC and to evaluate whether the cell suspension contained GC B and T cells expressing CD20, CD45RO, CD62L and CDw75. More than 90% of the lymphocytes outside the clusters were positive for CD20 and CDw75, and less than 5% were positive for CD45RO and CD62L. Therefore, the lymphocytes in the cell suspension were considered to be have come from the GC.

Isolation of FDC from GC

The GC enucleated from PLP-fixed tonsillar tissues were digested with collagenase (class IV, 450 U/ml, Sigma) and deoxyribonuclease I (28 kU/ml, Sigma) in PBS at 37°Cfor 1 h under gentle, constant stirring. The cell suspension was washed with PBS and passed through a 133μm nylon mesh. A discontinuous density gradient was prepared using Patho-o-cyte 5 (Miles Laboratories, Elkhart, IN, USA) with PBS adjusted to the densities 1.052 g/ml and 1.030 g/ml. The higher density Patho-o-cyte solution (6 ml) was placed in a centrifugation tube and overlaid with the lower density solution (4 ml). Four ml of the cell suspension (100–200 x 10^6 cells/ml) was layered onto the top of the tube and centrifuged at 8500g for 1 h. The interphase between the 1.052 and 1.030 g/ml solutions was collected. Cells were washed with PBS and incubated with anti-

FDC antibody (Ki-M4, mouse IgG2a ; Biomedicals AG, Rheinstrasse, Switzerland) overnight at 4°C. The cell preparation was then washed with PBS and incubated with dynabeads coated with sheep anti-mouse IgG antibody (Dynal A. S., Oslo, Norway) for 1 h at 4°C on the rotary mixer. The cell preparation was held stationary for 1 min under a magnetic field (MPC -1, Dynal A. S.). Supernatant containing unlabelled cells was removed, while cells coated with dynabeads were retained and then incubated with chymopapain (200 pKat/0.12 U/ml, Sigma) for 20 min at 37°C in a rotary mixer. They were then held again under a magnetic field 2 min. Supernatant containing the purified FDC was collected and centrifuged at 1000 g for 5 min. Cytospin slides were prepared for immunocytochemical double staining.

Immunohistochemistry

Lymphoid tissues and diseased tissues were stained using immunohistochemical techniques. The avidin-biotin immunoperoxidase complex (ABC) method was performed. PLP-fixed 5μm cryostat sections were incubated for 45 min at 4°Cwith 0.3% hydrogen peroxide in methanol to block the endogenous peroxidase activity, and then with 10% skim milk (Snow Brand, Sapporo) in PBS for 15 min. The sections were incubated with anti-HSP72/73 antibody overnight at 4°Cin a humidified chamber. After washing, biotinylated F (ab')$_2$ fragments of affinity-isolated rabbit anti-mouse immunoglobulins (DAKO) were applied for 2 h, followed by application of ABC complex (Vectastain, ABC Elite ; Vector Laboratories, CA, USA) for 30 min. Samples were then treated with the chromogen, 3,3'-diaminobenzidine tetrahydrochloride (DAB ; Dojindo Chemicals, Kumamoto). Finally, the sections were counterstained with methyl green.

As a negative control, sections prepared by serial sectioning were stained according to the same procedure using PBS or mouse IgG1, IgG2 or IgM (DAKO) in stead of the primary antibody.

The immunoreaction was graded as negative (−), partially positive (+/−), weakly to moderately positive (+), and strongly positive (++). The secondary LF were divided into five zones : the mantle zone, outer zone, apical light zone, basal light zone, and dark zone. The margin of each zone was decided based on the light microscopic findings of hematoxylin and eosin staining and CD23 immunostaining of serial PLP-fixed sections.

Immunohistochemical and immunocytochemical double staining

For immuno- chemical double staining, the first ABC method staining was visualized with 3-amino-9-ethylcarbazole (AEC) to identify HSP72/73. Tissue section- and cytospin-slides were t hen immersed in 0.2 M glycine-HCl for 2 h to elute the primary antibody, before staining by the immunoalkaline-phosphatase method to identify FDC ; tissue section- and cytospin-slides were incubated with anti-FDC antibody (R4/23) overnight at 4°C, and, after washing, alkaline-phosphatase conjugated F (ab')$_2$fragments of affinity-isolated goat anti-mouse IgM (H+L) (American Qualex, San Clemente, CA, USA) were applied for 2 h. The chromogen, 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium chloride (BCIP/NBT) were then added in the dark under microscopic control.

Immunoelectron microscopy

The PLP-fixed tonsillar specimens cut into 50μm-thick slices with a microslicer were used for the indirect immunoperoxidase method. The sliced specimens were immersed in bovine serum albumin (Sigma) for 2 h at 4°C, and then anti-HSP72/73 antibody was applied for 24 h at 4°C. After washing, the specimens were incubated with horseradish peroxidase-conjugated F (ab')$_2$ fragments of affinity-isolated rabbit anti-mouse IgG (H+L) (American Qualex) for 2 h at 4°C, and the staining was visualized with DAB. After refixing with 1.25% glutaraldehyde for 30 min and 2% osmium tetroxide for 2 h at 4°C, the specimens were dehydrated through graded alcohols and embedded in epoxy resin. Ultra thin sections were prepared using an ultramicrotome (Porter-Blum, Sorvall, CT, USA), and observed under a Zeiss EM109 electron microscope (Carl Zeiss, Oberkochen, Germany).

In situ hybridization

A complementary DNA probe[23] for the human heat shock 70 kD protein 1 purchased from American Type Culture Collection (Rock-

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Table 1. HSP72/73 expression in the tonsillar secondary lymphoid follicle

Follicular zone	Staining intensity	Follicular dendritic cells		Lymphocytes		Vascular wall
		Cytoplasm	Nucleus	Cytoplasm	Nucleus	
Mantle zone	Low	+	+/−	+	+/−	+
Outer zone	Low	+	+/−	−	−	+
Apical light zone	High	++	+	−	−	+
Basal light zone	High	++	+	−	−	+
Dark zone	Low	+	+/−	−	−	+

− ; negative, +/− ; partially positive, + ; weakly to moderately positive, ++ ; strongly positive

ville, MD, USA) was labeled with digoxigenin using a Nick Translation Kit (Boehringer Mannheim GmBH, Mannheim, Germany).

PLP-fixed 5μm-thick cryostat sections were baked overnight at 55°C. The sections were rehydrated in PBS for 15 min, immersed in 0.2 M HCl for 10 min, and incubated in 10μg/ml proteinase-K in 100 mM Tris, pH 8.0, 50 mM EDTA at 37°Cfor 10 min. After three 5 min washes in PBS to arrest proteolysis, sections were immersed in 4% paraformaldehyde for 10 min, followed by two 15 min washes in 0.1 M glycine in PBS. Sections were dehydrated through graded alcohols and air-dried. Hybridization mixture (50μl) containing digoxigenin-labeled DNA probe at a final concentration of 1.0μg/μl hybridization buffer (Intron Lab. Inc., Tokyo, Japan) was boiled for 10 min and cooled quickly. The mixture was applied to the sections and covered with individual siliconized coverslips and incubated in a humidified chamber overnight at 55°C. Following hybridization, the coverslips were removed in 2 x SSC (1 x SSC : standard saline citrate, 0.15 M sodium chloride, 0.03 M sodium citrate), and excess probe was removed by thorough washing in 50% formamide 2 x SSC twice for 30 min each at 45°C, 2 x SSC twice for 15 min each at 45°Cand 0.2 x SSC twice for 15 min each at room temperature. Immunological detection was performed using a Digoxigenin Detection Kit (Boehringer Mannheim Biochemical). In brief, sections were washed in buffer 1 (100 mM Tris-HCl, 150 mM NaCl, pH 7.5), followed by incubation in buffer 1 containing 0.5% blocking reagent for 30 min. After washed in buffer 1, alkaline-phosphatase conjugated polyclonal sheep anti-digoxigenin Fab fragment antibody diluted 1 : 1000 in buffer 1 was applied to each section, and incubated in a humidified chamber for 1 hr. After washing in buffer 1, sections were immersed in buffer 3 (100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl2, pH 9.5). A color reaction was performed with BICP/NBT in the dark under microscopic control. The reaction was stopped in distilled water and the sections were mounted in Crystal-Mount (Cosmo BioChemicals, Tokyo).

As a negative control, sections were hybridized with hybridization buffer instead of cDNA probe by the same procedure.

As a positive control, sections were hybridized with fluorescein isothiocyanate (FITC) labeledα-actin probe, followed by alkaline-phosphatase conjugated anti-FITC antibody and BICP/NBT as a substrate.

RESULTS

Immunohistochemistry

The results of HSP72/73 immunostaining in each of the five follicular zones are summarized in Table 1. HSP72/73 was detected on any FDC, showing a reticular network pattern in the secondary LF (Fig. 1A, 1B, 1C & 1D). The light zone was stained more intensely than other zones (Fig. 1C), while the dark zone was sparsely stained (Fig. 1D). Not only the cytoplasm, but also the nuclei of FDC were diffusely labeled. However, HSP72/73 was not detected on any lymphocytes in the GC. The cytoplasm of some MZ lymphocytes was also labeled faintly (Fig. 1 B).

After immunohistochemical double staining, HSP72/73 visualized with AEC was present throughout the cytoplasm and in the nuclei of FDC with cell surfaces colored by anti-FDC antibody (R4/23) and BCIP/NBT (Fig. 2). In addition, a dotted reaction was stressed in the nuclei, corresponding to expression in the nucleoli.

There were no marked differences in HSP72/73 positivity in the secondary LF between tonsillar tissues and other lymphoid tissues,

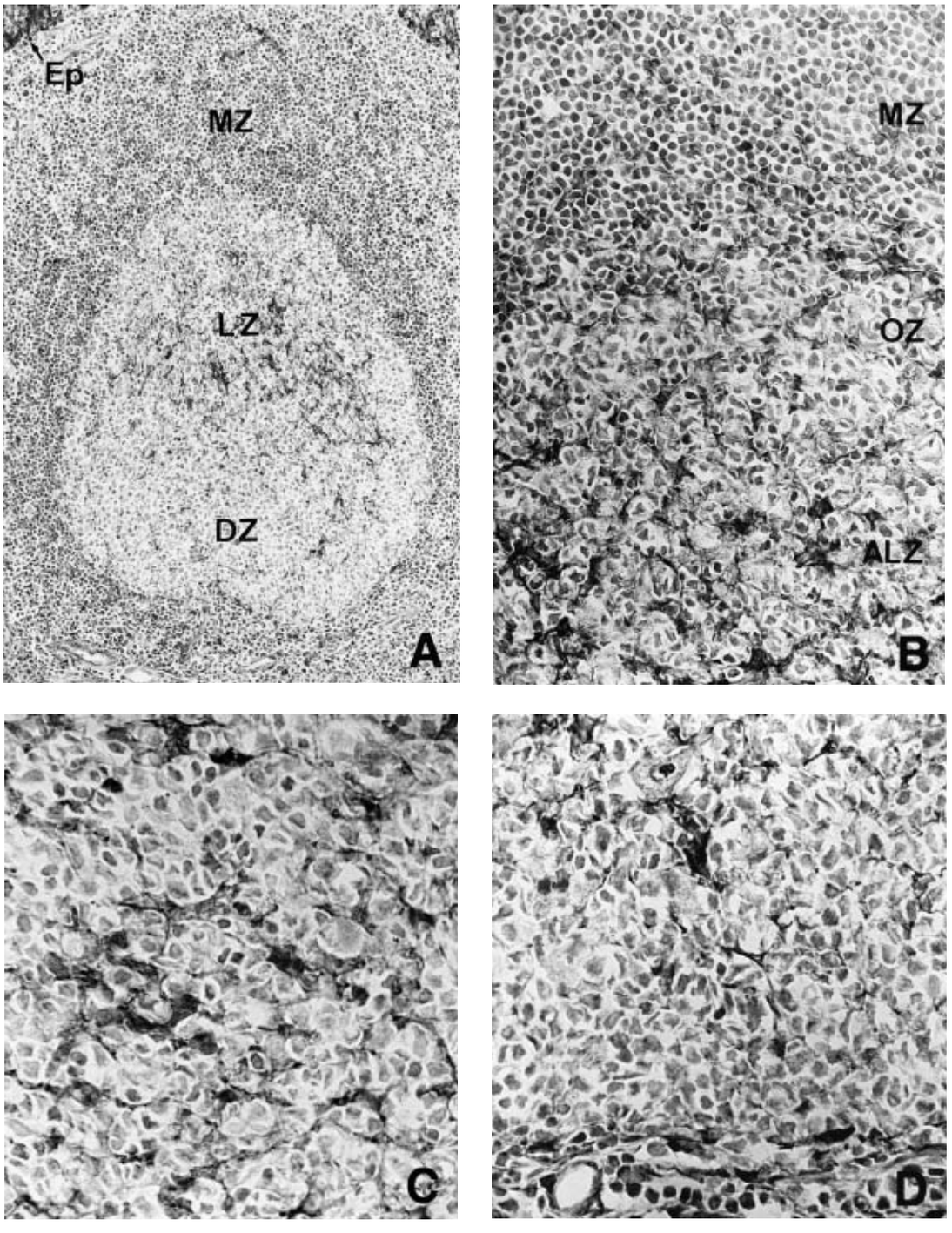

Fig. 1. Immunostaining of HSP72/73 in a tonsillar lymphoid follicle (LF). (A) High power view of a secondary LF. A positively-labeled reticular meshwork was seen in the LF, and the light zone (*LZ*) was intensely labeled, while the positive reaction was relatively sparse in the dark zone (*DZ*). The mantle zone (*MZ*) was faintly labeled. Tonsillar epithelium (*Ep*) was densely labeled. (B) High power view of the mantle zone (*MZ*), outer zone (*OZ*) and apical light zone (*ALZ*). MZ lymphocytes were labeled faintly. Cells in the OZ and ALZ were positive in the reticular pattern. (C) High power view of the basal light zone. Note the extensively labeled reticular meshwork pattern. No distinct positive reaction was found on any germinal center lymphocytes. (D) High power view of the dark zone. The positive reaction was relatively sparse in this zone. Counterstained with methyl green. (Original magnification (A) 120x, (B) 640 x, (C) 640 x, (D) 640 x)

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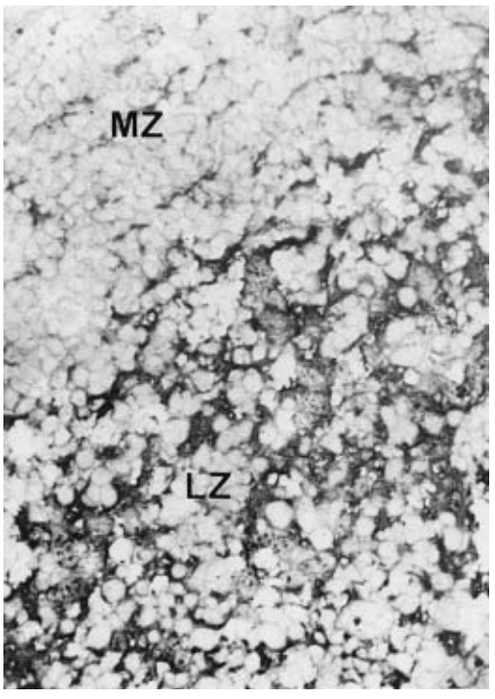
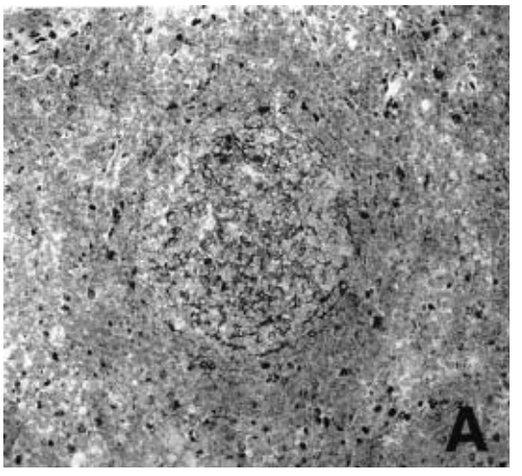
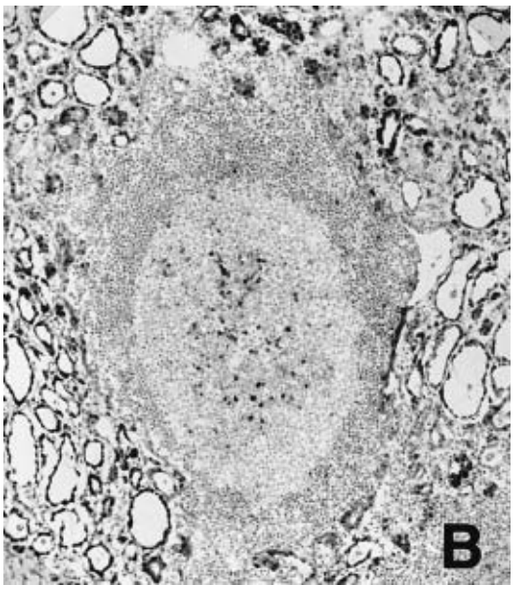


Fig. 2. Double immunostaining for HSP72/73 and R4/23 in a tonsillar lymphoid follicle. FDC colored with BCIP/NBT (black) showing a reticular meshwork in the light zone (*LZ*) and mantle zone (*MZ*) that simultaneously express HSP72/73, visualized with AEC (red) in the cytoplasm and nucleus. Note the dotted reaction in the nuclei. (Original magnification 400 x)





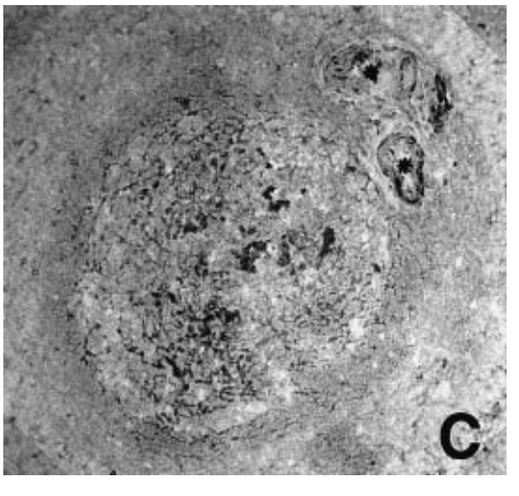


Fig. 3. Immunostaining of HSP72/73 in lymphoid follicles (LF) in neck lymph node (A), Hashimoto's thyroiditis (B) and spleen (C). Similar to tonsillar LF, HSP72/73 was detected in germinal centers, showing a reticular meshwork pattern, and faintly in the mantle zone. Scattered positive cells are found in the paracortex and lymphatic sinuses (A). A positive reaction is also found on thyroid follicular epithelium surrounding LF (B) and splenic vascular wall (asterisk) (C). Counterstained with methyl green. (Original magnification (A & C) 180 x, (B) 120 x)

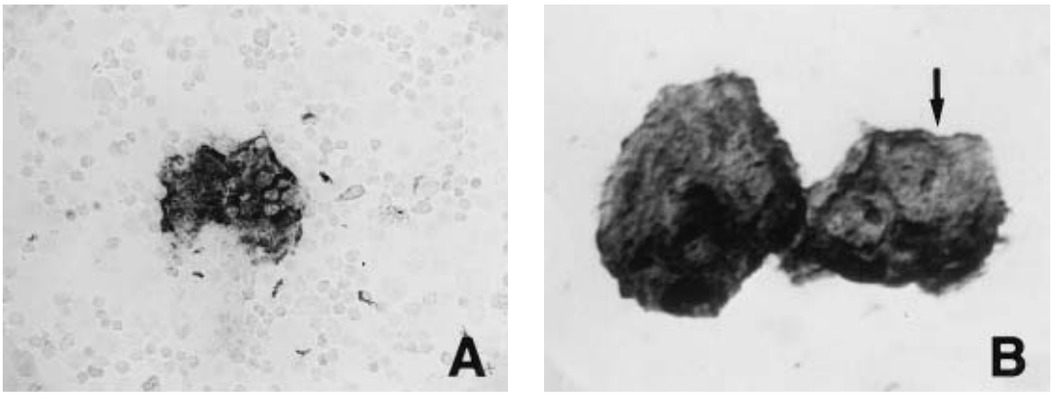


Fig. 4. Double immunostaining for HSP72/73 and R4/23 in an FDC-associated lymphocyte cluster (A) and in an isolated FDC (B). Red colored FDC with anti- HSP72/73 and AEC simultaneously express R4/23, visualized with BCIP/NBT (black). The lymphocytes in and outside the clusters in the GC were not labeled (A). A binucleated FDC (arrow) expresses HSP72/73 in the cytoplasm and nucleus (B). (Original magnification (A) 730 x, (B) 960 x)

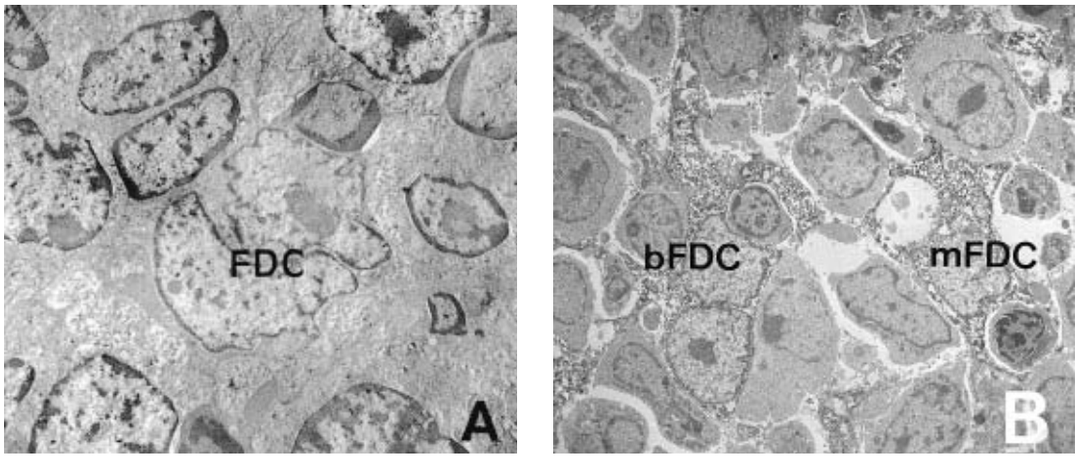


Fig. 5. Immunoelectron microscopy of HSP72/73 in the tonsillar light zone. (A) Negative control replacing the primary antibody with PBS. (*FDC*) follicular dendritic cell. (B) The cytoplasmic cytosol of mononuclear (*mFDC*) and binucleated (*bFDC*) FDC is diffusely labeled. The nuclei of FDC are faintly labeled with a dotted nucleolus. Note the absence of distinct staining of the surrounding lymphocytes. (Original magnification A ; 3600 x, B ; 2400 x)

although some minor differences in the intensity and distribution of the immunoreactivity were observed (Fig. 3A, 3B & 3C).

Immunocytochemistry

Immunocytochemical double staining showed that HSP72/73 was present within FDC-associated lymphocyte clusters, with simultaneous and extensive labeling of the cytoplasmic extensions of FDC with R4/23 (Fig. 4A). However, the lymphocytes in and outside the clusters in the GC were not labeled. HSP72/73 was detected in the cytoplasm and nucleus of isolated FDC (Fig. 4B).

Immunoelectron microscopy

Immunoelectron microscopy revealed that HSP72/73 was diffusely and evenly distributed in the cytoplasm and in the nuclei of FDC, but all GC lymphocytes were negative for HSP72/73 (Fig. 5A & 5B). Nuclear dotted staining in FDC indicated nucleolar staining.

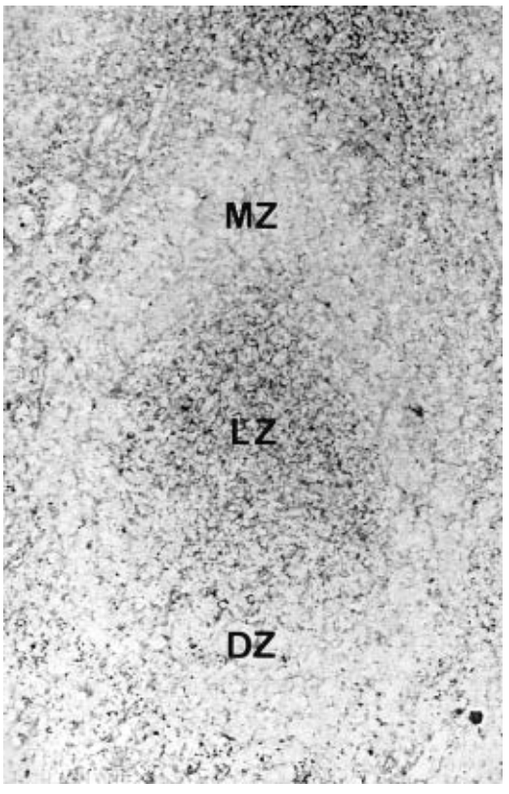


Fig. 6. *In situ* hybridization of HSP70 mRNA in a tonsillar lymphoid follicle. Intense signaling visualized with BCIP/NBT is detected in the lymphoid follicle, especially in the light zone (*LZ*). The reaction is negative or faint in the mantle zone (*MZ*) and dark zone (*DZ*). There is additional reticular staining in the extrafollicular area above the mantle zone, although its significance is not clear. (Originalmagnification 120x)

Table 2. *In situ* hybridization of HSP70 mRNA in the tonsillar secondary lymphoid follicle

Follicular zone	Staining intensity	Staining pattern
Mantle zone	Low	Loose reticular pattern
Outer zone	Low	Loose reticular pattern
Apical light zone	High	Dense reticular pattern
Basal light zone	High	Dense reticular pattern
Dark zone	Low	Sparsely reticular pattern

In situ *hybridization*

HSP70 mRNA was detected as a reticular meshwork in the cytoplasm of cells in the LF and revealed denser labeling of the light zone than of other follicular zones (Fig. 6), which is similar to the staining pattern of its protein. Almost all follicular lymphocytes, including those in the mantle zone, were negative. Some vascular walls in the GC were faintly positive for HSP70 mRNA (Table 2).

DISCUSSION

In all the LF examined in the present study, HSP72/73 was detected in the cytoplasm and nuclei of FDC, showing denser staining predominantly in the light zone, whereas the lymphocytes were HSP72/73 negative. Dense staining of the light zone may have been due to the higher frequency of FDC in this zone, compared to the other zones. The follicular localization and staining intensity of HSP72/73 were similar among the lymphoid tissues examined, although some minor differences were recognized. These differences may include the relatively difficul preparation of tissue to simultaneously reveal five follicular zones in the same section. It should be stressed that antigen-stimulated LF are the professional site of generation of antigen-specific memory B cells and plasmablasts[18,19]. The present study, for the first time, has demonstrated that the inducible and constitutive stress proteins HSP72/73 and HSP70-m RNA were ubiquitously expressed in the LF and were almost exclusively expressed by FDC in the GC.

There are no previous reports describing the subcellular localization of HSP72/73 on FDC. The present light- and electron-immunochemical results demonstrated a diffuse cytoplasmic distribution of HSP72/73 in the FDC cell body and, in addition, a dotted nuclear pattern of HSP72/73 staining was seen. The latter indicates that the HSP72/73 protein localizes in the nucleoli, which is thought to occur in response to some stimuli or stresses. HSP70 that is usually present in the cytoplasm is translocate and accumulates in the nuclei and especially in the nucleoli during stress, and then gradually returns to the cytoplasm after the stress is relieved. HSP70 associates with partially denatured proteins and protein aggregates in the nuclei, repairs them and then dissoci-

ates from them[24].

HSP overexpressed by stress protects cells against stress-induced apoptosis, although it is unclear whether HSP itself play a role in preventing apoptosis. HSP as molecular chaperone binds to denatured, misfolded or aggregated proteins and subsequently assist the proteins to renature, refold or be removed. Recent studies indicate that HSP may affect the agents of regulating apoptosis, the inflammatory cytokines, including tumor necrosis factor-α[25], interleukins-1, -6, and-10[26,27], and nuclear factor-kappa B[28,29]. The latter is expressed on FDC[30]. FDC are more resistant to stresses, including irradiation, than GC lymphocytes[31] and, in fact, apoptosis of FDC has never been observed. Furthermore, it is stressed that some HSP are essentialfor cell viability under normal conditions of growth[8,13].

HSP70 family proteins transport the degraded antigen to the rough endoplasmic reticulum in the antigen-presenting cells (APC)[32-34]. Two members of the HSP70 family, BiP and PBP72/74, relate to the expression of MHC molecules. FDC, however, do not internalize and process antigen. It is speculated that exogenous antigens, such as the HSP70-peptide complexes, may be taken-up by APC either non-specifically or possibly through a receptor specific for the HSP[35,36]. In GC, B lymphocytes may take up the HSP70-immune-complexes. Although HSP72/73 may play a role as a chaperone in FDC, the immunological relation between HSP72/73 and FDC is unclear. The precise significance of HSP72/73 expression on FDC still remains a mystery.

We demonstrate here that FDC ubiquitously express the protein and mRNA of HS P72/73 in all lymphoid tissues, whereas GC lymphocytes are HSP72/73 negative, suggesting that HSP72/73 expression is specific for FDC, except vascular walls within the GC. Moroi Y, et al.[37] have recently described the induction of cellular immunity by immunization with novel hybrid peptides complexed to HSP 70. The present study may provide some suggestions to the augmentation of FDC-related germinal center response with HSP 70 family.

REFERENCES

1 Hugh RBP : Function of the hsp70 protein family : An overview. In Stress Proteins in Biology and Medicine, Morimoto RI, Tisseieres A, Georgopoulos C (eds), Cold Spring Harbor Laboratory Press : New York, 1990 ; 287-322.

2 Craig EA : Chaperones : Helpers along pathway to protein folding. Science 260 : 190 21903, 1993

3 Ellis RJ, van der Vies SM : Molecular chaperones. Annu Rev Biochem 60 : 321-347, 1991

4 Gething MJ, Sambrook J : Protein folding in the cell. Nature 355 : 33-45, 1992

5 Becker J, Craig EA : Heat-shock proteins as molecular chaperones. Eur J Biochem 219 : 11-23, 1994

6 Gunther E, Walter L : Genetic aspects of the hsp70 multigene family in vertebrates Experimentia 50 : 987-1001, 1994

7 Taggart DP, Bakkenist CJ, Biddolph SC, Graham AK, McGee JO : Induction of myocardial heat shock protein 70 during cardiac surgery. J Pathol 182 : 362-366, 1997

8 Lindquist S, Craig EA : The heat-shock proteins. Annu Rev Genet22 : 631-677, 1988

9 Wells AD, Malkovsky M : Heat shock proteins, tumor immunogenicity and antigen presentation : an integrated view. Immunol Today 21 : 129-132, 2000

10 Schafer C, Williams JA : Stress kinase and heat shock proteins in the pancreas : possible roles in normal function and disease. J Gastroenterol 35 : 1-9, 2000

11 Yenari MA Giffard RG, Sapolsky RM, Steinberg GK : The neuroprotective potential of heat shock protein 70 (HSP 70). Mol Med Today 5 : 525-531, 1999

12 Youde SJ, Mower J, Moore DP, Parkes AB : Stress protein expression in primary and immortalized cultures of human thyroid cells : a model system for the study of stress proteins in the pathogenesis of autoimmune thyroid disease. Cell Stress Chap 3 : 89-93, 1998

13 Mallouk Y, Vayssier-Taussat M, Bonventre JV, Polla BS : Heat shock protein 70 and ATP as partners in cell homeostasis. Int J Mel Med 4 : 463-474, 1999

14 DeNagel DC, Pierce SK : A case for chaperons in antigen processing. Immunol Today 13 : 421-422, 1992

15 Groettrup M, Soza A, Kuckelkorn U, Kloetzel PM : Peptide antigen production by the proteasome : complexity provides efficiency. Immunol Today 17 : 429-435, 1996

16 Vanbuskirk A, Crump BL, Margoliash E, Pierce SK : A peptide binding protein having a role in antigen presentation is a member of the HSP70 heat shock family. J Exp Med 170 : 1799-1809,

1989
17 Grouard G, Durand I, Filgueira L, Banchereau J, Liu Y-J : Dendritic cells capable of stimulating T cells in germinal centres. Nature 384 : 364-367, 1996
18 Imai Y, Yamakawa M, Kasajima T : The lymphocyte-dendritic cell system. Histol Histopathol 13 : 469-510, 1998
19 Wu J, Qin D, Burton GF, Szakal AK, Tew JG : Follicular dendritic cell-derived antigen and accessory activity in initiation of memory IgG responses in vitro. J Immunol 157 : 3404-3411, 1996
20 Grouard G, de Bouteiller O, Barthelemy C, Lebecque S, Banchereau J, Liu YJ : Regulation of human B cell activation by follicular dendritic cell and T cell signals. Curr Top Microbiol Immunol 201 : 105-117, 1995
21 Tew JG, Wu J, Qin D, Helm S, Burton GF, Szakal AK : Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. Immunol Rev 15 639-52, 1997
22 Tsunoda T, Yamakawa M, Takahashi T : Differential expression of Ca2+-binding proteins on follicular dendritic cells in non-neoplastic and neoplastic lymphoid follicles. Am J Pathol 155 : 805-814, 1999
23 Lawrence JB, Singer RH : Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. Nucleic Acids Res 13 : 1777-1799, 1985
24 Lewis MJ, Pelham HRB : Involvement of ATP in the nuclear and nucleolar functions of the 70 kd heat shock protein. EMBO J 4 : 3137-3143, 1985
25 Kim YM, de Vera ME, Watkins SC, Billiar TR : Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor--induced apoptosis by inducing heat shock protein 70 expression. J Biol Chem 272 : 1402-1411, 1997
26 Hall TJ : Role of hsp70 in cytokine production. Experimentia 50 : 1048-1053, 1994
27 Arai T, Hiromatsu K, Nishimura H, Kimura Y, Kobayashi N, Ishida H, Nimura Y, Yoshikai Y : Endogenous interleukin-10 prevent apoptosis in macrophages during Sal monella infection. Biochem Biophys Res Com 213 : 600-607, 1995
28 DeMeester SL, Buchman TG, Qiu Y, Jacob AK, Dunnigan K, Hotchkiss RS, Karl I, Cobb JP : Heat shock induces IB- and prevents stress-induced endothelial cell apoptosis. Arch Surg 132 : 1283-1288, 1997
29 Franzoso G, Carlson L, Poljak L, Shores EW, Epstein S, Leonardi A, Grinberg A, Tran T, Scharton-Kersten T, Anver M, Love P, Brown K, Siebenlist U : Mice deficient in nuclear factor (NF)-kappa B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture. J Exp Med 187 : 147-159, 1998
30 Feuillard J, Korner M, Fourcade C, Costa A, Binet J-L, Debre P, Raphael M : Visualization of the endogenous NF-B p50 subunit in the nucleus of follicular dendritic cells in germinal centers. J Immunol 152 : 12-21, 1994
31 Gordon SA, Hoffman RA, Simmons RL, Ford HR : Induction of heat shock protein 70 protects thymocytes against radiation-induced apoptosis. Arch Surg 132 : 1277-1282, 1997
32 Melnick J, Argon Y : Molecular chaperons and the biosynthesis of antigen receptors. Immunol Today 16 : 243-250, 1995
33 Arnold D, Wahl C, Faath S, Rammensee HG, Schild H : Influences of transporter associated with the endoplasmic reticulum-resident stress protein gp96. J Exp Med 186 : 461-466, 1997
34 Pierce SK : Molecular chaperones in the processing and presentation of antigen to helper T cells. Experimentia 50 : 1026-1030, 1994
35 Roman E, Moreno C : Synthetic peptides noncovalently bound to bacterial hsp70 elicit peptide-specific T-cell responses in vivo. Immunology 88 : 487-492, 1996
36 Ciupitu AM, Petersson M, O'Donnell CL, Williams K, Jindal S, Kiessling R, Welsh RM : Immunization with a lymphocytic choriomeningitis virus peptide mixed with heat shock protein 70 results in protective antiviral immunity and specific cytotoxic T lymphocytes. J Exp Med 187 : 685-691, 1998
37 Moroi Y, Mayhew M, Trcka J, Hoe MH, Takechi Y, Hart FU, Rothman JE, Houghton AN : Induction of cellular immunity by immunization with novel hybrid peptides complexed to heat shock protein 70. Proc Natl Aca USA 97 : 3485-3480