Expression of Ro/SS-A and La/SS-B determined by immunohistochemistry in healthy, inflamed and autoimmune diseased human tissues: a generalized phenomenon

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Abstract

Objective

Autoantibodies to the ribonucleoproteins Ro/SS-A and La/SS-B are found in autoimmune diseases such as primary Sjögren's syndrome (pSS), systemic lupus erythematousus and rheumatoid arthritis. Increased and aberrant expression of Ro/SS-A and La/SS- B in target organs, which have been reported in the recent literature, might contribute to their antigenicity. However, data on the expression of Ro/SS-Aand La/SS-B in other inflammatory conditions are scarce.

Materials and methods

Using monoclonal antibodies against Ro/SS-A and La/SS-B, we studied the expression of these antigens in paraffin-embedded healthy tissue, aspecific inflamed tissue, the neonatal and adult cardiac conduction systems and labial salivary gland tissues of patients suspected of having pSS.

Results

In healthy tissues, the nuclei expressed both Ro/SS-A and La/SS-B. This expression was stronger in inflamed tissues. Nucleoli were negative and cytoplasmic expression was weaker than nuclear expression. No increased or aberrant expression of Ro/SS-A or La/SS-B was observed in either neonatal or adult atrioventicular nodes and bundle branches. More pSS patients showed high La/SS-B immunoreactivity levels in their labial salivary gland ductal cell nuclei than non-Sjögren's syndrome sicca patients.

Conclusions

Ro/SS-A and La/SS-B expression is a generalized cell biological phenomenon and may be upregulated by increased cell activation both in aspecific and autoimmune-mediated inflammation. In pSS the high expression of La/SS-B in labial salivary gland ductal cell nuclei might contribute to the local immune response.

Key words

Ro/SS-A, La/SS-B, Sjögren's syndrome, immunohistochemistry, congenital heart block.

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Introduction

Autoantibodies to the ribonucleoproteins (RNP) Ro/SS-A and La/SS-B are found in autoimmune diseases as Sjögren's syndrome (SS), systemic lupus erythematousus (SLE) and rheumatoid arthritis. Maternal anti-Ro/SS-A antibodies have been correlated with congenital heart block (CHB) in neonates born to anti-Ro/SS-A positive mothers (1-3). Moreover, there is mounting evidence that in SS salivary glands anti-Ro/SS-A and anti-La/SS-B antibodies are produced locally (4, 5).

Several reports have shown increased expression of Ro/SS-Aand La/SS-B antigens in autoimmune diseased target organs. For example, a 4- to 10-fold higher expression of Ro/SS-A and La/SS-B was shown in skin biopsy specimens obtained from patients suffering from SLE with photosensitivity compared to SLE patients without photosensitivity (6). Tzioufas *et al*. showed the upregulation of La/SSB mRNA in acinar and mononuclear cells in the minor salivary glands of primary Sjögren's syndrome (pSS) patients with anti-La/SSB antibodies (7). Moreover, the 'aberrant'expression of La/SS-B (i.e. not only expression in the nucleoli of acinar cells, but also in nucleo- and cytoplasm as well as along the cell membrane) in frozen sections of labial salivary gland biopsy specimens of SS patients was reported by de Wilde *et al*. (8).

It has been shown that apoptosis may induce the surface expression of autoantigens including Ro/SS-A and La/SS-B (9). This could explain earlier findings stating that viral infection of cells *in vitro* is able to induce the redistribution of La/SS-B to the cytoplasm and induce the surface expression of La/SS-B (10).

La/SS-B RNP has a molecular weight of 48 kD, contains a RNPconsensus motif and functions as a termination factor for RNA polymerase III (11). Ro/SS-A RNPs are composed of several proteins complexed with a subset of La/SS-B associated RNAs, the Y-RNAs (12). In human cells four different Y-RNAs have been identified, together with two different Ro/SS-A proteins, designated Ro60 and Ro52 (13-15) based on their molecular weight. No function as yet has been associated with either of these two Ro/SS-A proteins, although the overexpression of Ro52 has been reported to enhance IL-2 production in T cells via the CD28 pathway (16).

The aforementioned increased or aberrant expression of Ro/SS-Aand La/SS-B RNPs in autoimmune diseased target organs might contribute to their antigenicity. However, data on the expression of Ro/SS-A and La/SS-B in other inflammatory conditions are scarce. The present study was undertaken to investigate whether the increased or aberrant expression of Ro/SS-Aand/or La/SS-B is specific for autoimmune diseases such as CHB and pSS. Therefore, we studied the expression of Ro/SS-A and La/SS-B using immunohistochemistry on paraffin-embedded healthy tissue, aspecific inflamed tissue, the neonatal and adult cardiac conduction systems and labial salivary gland tissues of patients suspected of having pSS.

Materials and methods

Materials

The expression of Ro/SS-Aand La/SS-B in normal, non-inflamed human tissues (obtained at autopsies) was investigated in the liver (n = 5), submandibular salivary gland (n = 2), pancreas (n = 2), thyroid gland (n = 1), adrenal gland (n = 1), brain stem (pons) (n = 3), cerebrum (n= 3), small (n= 2) and large intestine (n = 2), appendix (n = 1), kidney (n= 1), tonsil (n= 5), spleen (n = 1), testis (n = 1), prostate (n = 1), lung (n = 1) and bone marrow (n=2).

The expression of Ro/SS-Aand La/SS-B in inflamed tissue (obtained after surgical procedures) was studied in the appendix with acute inflammation (n = 2), submandibular salivary gland and prostate with aspecific chronic inflammation (n=2 and n=1 respectively), lymph node with granulomatous inflammation due to tuberculosis (n = 1) and perineal skin with fistular inflammation (n=1).

With respect to the expression of Ro/SS-A and La/SS-B in the cardiac conduction system (atrioventricular node and bundle branches) adult heart (n= 2) and neonatal heart (n = 4) tissue specimens (obtained at autopsies) were examined.

To study the expression of Ro/SS-A and La/SS-B in salivary glands in pSS, labial salivary gland biopsy specimens from 41 sicca patients suspected of having SS were used. According to recently developed American-European criteria (17), 15 of the the patients were classified as suffering from pSS; 26 patients did not fulfill these criteria and were consequently classified as non-SS (nSS). The expression in both acinar and ductal cell nuclei was investigated.

Tissue preparation

Tissues were fixed for 24-48 hours in phosphate buffered formalin (pH 7.2) and routinely embedded in paraffin. The bone marrow core biopsies were decalcified with buffered formic acid after fixation and paraffin embedded.

Immunohistochemistry

Two anti-Ro/SS-A mouse monoclonal antibodies (MoAbs) were used: MoAb 1D8 against Ro60 and MoAb 17D6 against Ro52. The anti-Ro52 MoAb was only used for the incubation of sections of healthy adult and neonatal heart tissue and of healthy adult liver, tonsil and salivary gland. Two anti-La/SS-B MoAbs (SW5.8 and 7F6) were used. Data on the properties of these MoAbs have been published previously (18,19). The MoAbs recognize epitopes on their antigens both in the free and complexed forms (e.g., they precipitate the relevant RNP particles). Optimal dilution of the MoAbs was determined by titration on both frozen and paraffin embedded healthy adult human liver sections (Table I).

The paraffin sections were stained as described previously (20) with the streptavidin-biotin-peroxidase technique. Briefly, 4 μm paraffin sections were heated for 15 minutes at 100ºC in 10 mmol/L sodium buffer (pH 6.0) to achieve antigen retrieval. The sections were allowed to cool slowly to room temperature and incubated according to the streptavidin-biotin-peroxidase technique. Peroxidase activity was visualized with 3,3'-diaminobenzidine tetrahydrochloride. Counterstaining was performed with Mayer's haematoxylin. Sections incubated with phosphate buffered saline instead of a specific MoAb served as negative controls; paraffin sections of healthy human liver incubated with the MoAbs served as positive controls.

Table I. Monoclonal antibodies used in immunohistochemistry.

Monoclonal antibody	Clone	Dilution	Reference
Anti-60 kD Ro/SS-A	1D8	0.025 μg/ml	Veldhoven *et al.* (18)
Anti-52 kD Ro/SS-A	17D6	0.15 μg/ml	Veldhoven *et al.* (18)
Anti-48 kD La/SS-B	SW5.8	1:20	Smith *et al.* (19)
Anti-48 kD La/SS-B	7F6	1.8 μg/ml	Veldhoven *et al.* (18)

Analysis of immunohistochemical data

The neonatal cardiac sections were independently scored by two observers (TMV and PGJN), all others by TMV and JMvW. The number of immunoreactive nuclei was classified as 'low'(0-25% immunoreactive nuclei), 'intermediate' (26-75% immunoreactive nuclei) or 'high' (76-100% immunoreactive nuclei). The chi-square test was used to determine differences in the distribution of the 3 immunoreactivity level groups between nSS and pSS patients.

Results

Expression of Ro/SS-A and La/SS-B in healthy human tissues

Ro/SS-A and La/SS-B immunostaining was observed in routinely fixed and paraffin-embedded tissues. The intensity of staining was stronger with anti-Ro60 MoAb than with anti-Ro52 MoAb and the two anti-La/SS-B MoAbs. The nuclei and overall constituents were adequately distinguishable using light microscopy. Negative nucleoli (blue-stained by haematoxylin counterstaining) in immunoreactive brown nucleoplasm could be observed in the neurons, ganglion cells, some mesenchymal cells in the neonatal heart, and hepatocytes (data not shown). Immunoreactive nucleoli were not seen in otherwise non-immunoreactive nuclei. In Table II the results of the immunostaining of healthy human tissues are summarized.

Liver: The hepatocytic nuclei showed both antigens in a moderate percentage. In some large immunoreactive nuclei no immunostaining of the nucleolus was observed (Fig.1). In hepatocytes containing strong nuclear expression of Ro/SS-A, weak cytoplasmic immunoreactivity for Ro/SS-Awas found. This phenomenon was predominantly present in areas where nearly all hepatocytes showed nuclear Ro/SS-A immunoreactivity. In these particular areas the nuclei of the sinusoidal lining cells, both Kupffer cells and endothelium also were immunoreactive (data not shown). Bile duct epithelial nuclei and cytoplasm were immunoreactive in a moderate percentage of the cells, although the cytoplasm only weakly.

Submandibular salivary gland: In general, ductal epithelial cell nuclei showed a high percentage of immunoreactivity for both antigens Ro/SS-Aand La/SS-B (Fig.2). A low percentage of the nuclei situated at the base of the acinar cells and densely compressed to the basement membrane were immunoreactive (Fig. 2).

Pancreas: Ductal epithelial nuclei showed Ro/SS-A and La/SS-B immunostaining in a high and moderate percentage, respectively. We observed weak ductal cytoplasmic Ro/SS-A expression. The endocrine cell nuclei of the islets of Langerhans were immunoreactive in a low percentage.

Thyroid gland: The thyrocytes showed nuclear expression of both proteins in a high percentage.

Adrenal gland: Cortical cell nuclei showed expression of both proteins although in a lesser percentage for La/SS-B than for Ro/SS-A. The medullary cell nuclei were immunoreactive in greater numbers for La/SS-B determined with MoAb SW5.8 than with MoAb 7F6. A reverse pattern was observed for the cortical cells with stronger expression of La/SS-B determined with MoAb 7F6 than with MoAb SW5.8.

Brain: The central nervous system offered us the opportunity to observe nucleoli in large neurons: the nucleoli were clearly negative for both proteins (Fig.

Table II. Ro/SS-Aand La/SS-B expression in healthy human tissues.

Tissue		Ro/SS-A1D8	La/SS-B 7F6	La/SS-B SW5.8
Liver	Hepatocytes	I	I	I
	Bile duct epithelium	I	I	I
	Sinusoidal lining cells	negative*	negative*	negative*
Submandibular gland	Ductal epithelium	H	H	H
	Acinar epithelium	L	L	L
Pancreas	Ductal epithelium	H	I	I
	Exocrine gland epithelium	I	I	I
	Islets of Langerhans	L	L	L
Thyroid gland	Follicular epithelium	H	H	H
Adrenal gland	Cortical cells	H	I	L
	Medullary cells	I	L	I
Pons	Arachnoidea	L	L	L
	Glial cells	H	I	I
	Neurons	I	L	L
	Ependymal cells	H	H	H
	Choroid plexus epithelium	L	L	L
Cerebrum-cerebellum	Arachnoidea	L	L	L
	Glial cells	H	I	I
	Neurons	L	L	L
Intestine	Epithelium	L	L	L
	Fibroblasts	I	I	I
	Smooth muscle cells	L	L	L
	Ganglion cells	H	L	L
Appendix	Epithelium	I	I	I
	Fibroblasts	L	L	L
	Smooth muscle cells	L	L	L
	Lymphoid cells	I	I	I
Kidney	Glomerular visceral epithelium	H	H	H
	Glomerular parietal epithelium	L	L	L
	Proximal tubular epithelium	H	I	I
	Distal/collecting tubular epithelium	H	H	H
Tonsil	Lining squamous epithelium	I	I	I
	B cell follicles	I	I	I
	Tlymphocytes	I	I	I
	Monohistiocytic cells	H	H	H
Spleen	Peri-arteriolar Tcells	I	I	I
	B cell follicles	I	I	I
	Sinusoidal lining cells	H	H	H
	Monohistiocytic cells	H	I	I
Testis	Sertoli cells	H	H	H
	Leydig cells	I	L	L
	Spermatogonia	H	H	H
	Spermatocytes, spermatids	L	L	L
	Spermia	L	L	L
Lung	Bronchial/bronchiolar epithelium	H	L	L
	Pneumocytes	L	L	L
Heart	Myocardium	L	L	L
	Endocardium	I	I	I
Bone marrow	Immature erythroid and myeloid cells	H	H	H
	Megakaryocytes	I	I	I
Striated muscle		L	L	L
Endothelium		I	I	I

The number of immunoreactive nuclei was classified as 'low' [L] (0-25% immunoreactive nuclei), 'intermediate' [I] (26-75% immunoreactive nuclei) or 'high'[H] (76-100% immunoreactive nuclei). See text for details.
*Negative except in area of 100% staining of hepatocytic nuclei.

3). The expression of Ro/SS-Awas stronger than of La/SS-B in the glial cell nuclei. Neurons showed immunostaining in a low percentage. The ependymal cell nuclei showed high immunostaining for both proteins. Arachnoidea and choroid plexus, however, showed no immunoreactivity.

Intestine: In the small and large intestine the epithelial cell nuclei expressed both antigens more intensely and in a higher percentage in the crypts than in the superficial layer. The nuclei of the appendix epithelium in the lamina propria expressed both proteins in a moderate percentage. There was low expression in the smooth muscle layers or in the fibroblasts. The ganglion cell nuclei of the mesenteric plexus were stained with the three MoAbs with here as well clearly non-immunoreactive nucleoli. The lymphoid cell nuclei in the appendix were immunoreactive in a moderate percentage.

Kidney: The glomeruli showed immunoreactivity of nearly all the visceral epithelial cell nuclei in contrast to the parietal cell nuclei which were mostly non-immunoreactive. The distal tubular nuclei showed Ro/SS-A and La/SS-B in nearly 100%.

Lymphoid organs: Tonsil and spleen showed a moderate to high expression of both antigens in all types of cells. The squamous epithelial lining over the tonsil expressed Ro/SS-Aand La/SS-B in a moderate percentage of the nuclei.

Testis: The basal cell nuclei of the seminiferous tubules were strongly immunoreactive for both proteins. The Leydig cell nuclei expressed both antigens in a low to moderate percentage.

Lung: Only the bronchial and bronchiolar cell nuclei showed Ro/SS-A positivity. The La/SS-B MoAbs revealed little immunoreactitvity.

Bone marrow: Immature erythroid and myeloid cells expressed both proteins in a high percentage. The megakaryocytic nuclei were immunoreactive in 50%; however, not all the nuclei in one megakayocyte appeared to be evenly stained or stained at all.

Striated muscle: In a low percentage of striated muscle cells, nuclear expression of both Ro/SS-A and La/SS-B was observed.

Endothelium: The nuclei in all of the investigated tissue specimens showed moderate immunoreactivity for both proteins.

To summarize, there appears to be a wide variation in the expression of Ro/SS-A and La/SS-B in normal, healthy human tissues. Nucleoli, when distinct, were negative.

Expression of Ro/SS-A and La/SS-B in inflamed human tissues

As shown in Table III a considerable percentage of the nuclei were positive for both Ro/SS-A and La/SS-B in inflamed human tissues. A remarkable finding was that endothelial and fibroblastic nuclei showed immunoreactivity in a higher percentage when located in areas of inflammation compared to non-inflamed areas.

In the acute appendicitis sections, all nuclei of the hyperplastic mesothelium over the inflamed area showed immunoreactivity for both Ro/SS-Aand La/SS-B (Fig. 4), whereas in a non-inflamed region of the same appendix the mesothelial cells were negative (Fig. 5).

The nuclei of the polynuclear giant (Langhans) cells in the tuberculous lymphadenitis sections were immunoreactive for both proteins (Fig. 6).

The epidermal cells in the fistular inflammatory skin were positive in all layers in a higher percentage of the nuclei than in the normal squamous epithelium over the tonsil. In the aspecific inflamed salivary gland sections 51-75% of the inflammatory (mainly lymphoid) cell nuclei were positive. Likewise, the inflammatory cell nuclei in the other inflamed tissues were positive in a high percentage for both proteins.

In summary, the expression of Ro/SS-A and La/SS-B appears to be linked to activation of cells in inflammatory foci.

Expression of Ro/SS-A and La/SS-B in cardiac tissue specimens

The adult heart biopsy specimens showed low immunoreactivity in the myocardial cell nuclei and intermediate immunoreactivity in endocardial cell nuclei for both antigens (Table II). The neonatal heart specimens showed immunoreactivity in about 11-25% of the myocardial nuclei and in 26-50% of the endocardial nuclei. The large mesenchymal cells present between the atrioventricular node cells showed faint cytoplasmic immunoreactivity for Ro/SS-A. The nuclei of these cells expressed both proteins in about 50%. The muscular node cells of the cardiac conduction system showed a similar distribution of both proteins as the muscular cells outside the node and bundle branches. No fixation of 52 kD or 60 kD Ro/SS-A MoAbs in the plasma membrane of the cardiac conductive system cells was observed neither in the adult nor in the neonatal heart specimens.

Expression of Ro/SS-A and La/SS-B in labial salivary gland biopsy specimens

The expression of both antigens in labial salivary glands was highly variable. In non-inflamed biopsy specimens the expression was weak. However, in inflamed salivary gland biopsy specimens (both from Sjögren's syndrome patients and 'aspecifically inflamed' labial salivary glands) moderate to strong expression was found in the inflamed areas (Fig.7); the ductal cell nuclei all showed immunostaining as did the nuclei of metaplastically changed acini (Fig.7). Furthermore, the endothelial cells as well as the fibroblasts showed nuclear immunostaining in these inflammatory foci. The mucous cell nuclei were intensely stained by haematoxylin, and possibly also by peroxidase activity in some (Fig.8). Rarely, nucleoli could be discriminated in the small dense nuclei as being non-immunoreactive.

The distribution of La/SS-B levels (MoAbs 7F6 and SW5.8) in acinar cell nuclei and ductal cell nuclei of nSS (n = 26) and pSS (n =15) patients are shown in Table IV. As can be observed, more pSS than nSS patients had high La/SS-B levels in their ductal cell nuclei. However, no significant differences in immunoreactivity levels for either 7F6 and SW 5.8 in the acinar cell nuclei could be found. Moreover, no differences in Ro/SS-A immunoreactivity could

Table III. Ro/SS-Aand La/SS-B expression in inflamed human tissues.

Tissue	Ro/SS-A1D8	La/SS-B 7F6	La/SS-B SW5.8
Acute appendicitis			
Muscularis mucosae/tunica muscularis	I	L	I
Lymphoid cells	H	I	H
Epithelium	H	I	H
Endothelium	H	H	H
Mesothelium	H	H	H
Chronic prostatitis			
Epithelium	I	I	H
Fibromuscular stroma	L	L	L
Lymphoid cells	L	L	L
Tuberculous lymphadenitis			
Lymphoid cells	H	H	H
Giant cells	H	H	H
Histiocytic cells	I	I	I
Endothelium	I	I	I
Perineal skin with fistular inflammation			
Adipocytes	L	L	L
Fibroblasts	H	H	H
Lymphoid cells	H	H	H
Keratinocytes	H	I	I
Aspecific sialadenitis			
Ductal epithelium	I	I	I
Acinar epithelium	I	I	I
Lymphoid cells	I	I	I

The number of immunoreactive nuclei was classified as 'low' [L] (0-25% immunoreactive nuclei), 'intermediate'[I] (26-75% immunoreactive nuclei) or 'high' [H] (76-100% immunoreactive nuclei). See text for details.

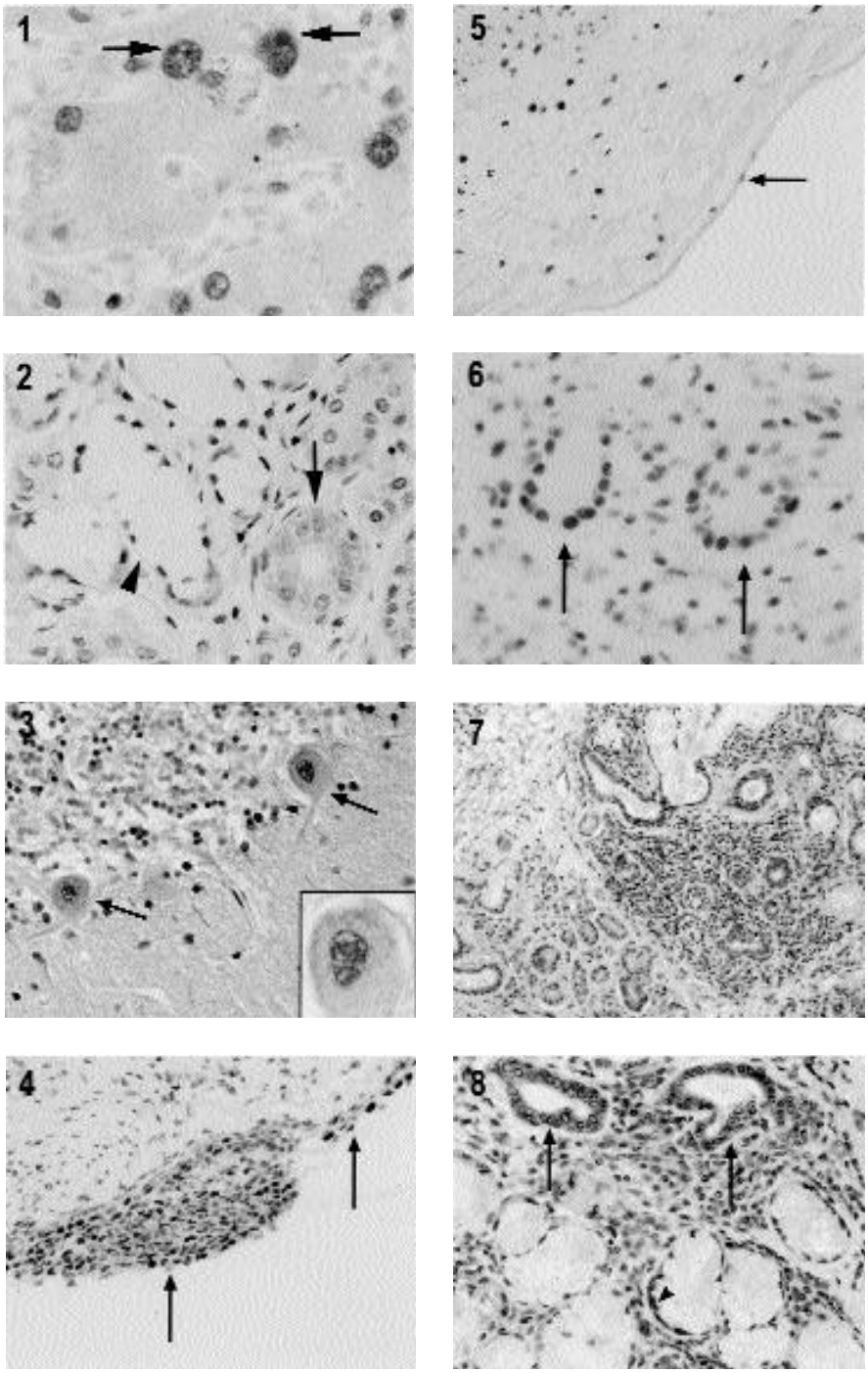

Fig. 1. Liver section stained for La/SS-B with MoAb 7F6: two large hepatocytic nuclei showing immunoreactive brown nucleoplasm and distinct negative blue nucleoli (**arrows**) can be seen (x 525).

Fig. 2. Healthy salivary gland section stained for La/SS-B with MoAb SW5.8 showing ducts with immunoreactive nuclei and weakly staining cytoplasm (**arrows**). The acini show dense faintly immunoreactive nuclei (**arrowheads**) (x 300).

Fig. 3. Cerebellar section stained for Ro/SS-A with MoAb 1D8. Two large immunoreactive neuronal nuclei can be seen (**arrows**) with negative nucleoli, as is highlighted in the inset (x 250 and x 675).

Fig. 4. Hyperplastic mesothelium lining an inflamed appendix (**arrows**) stained for La/SS-B with MoAb SW5.8. The mesothelial nuclei are all strongly positive. (x 200).

Fig. 5. Mesothelium stained for La/SS-B with MoAb SW5.8 lining a normal part of the same appendix, as shown in Figure 4. The mesothelial cell nuclei (**arrow**) are negative. Some nuclei in the submesothelial fatty tissue are positive (x 250).

Fig. 6. Two multi-nuclear Langhans cells (**arrows**) in a tuberculous lymphadenitis section stained for La/SS-B show strongly positive nuclei. Surrounding other inflammatory cells, lymphoid as well as epithelioid, show positive nuclei (x 350).

Fig. 7. Labial salivary gland section from a patient with Sjögren's syndrome stained for La/SS-B with MoAb SW5.8 showing both immunoreactive nuclei and cytoplasm of ductal cells. In the central part of the section the inflammatory cell nuclei are mostly positive. All acini are metaplastically changed by chronic inflammation and clearly express the antigen in the nuclei together with weak staining of the cytoplasm (x 125).

Fig. 8. Frozen SS labial salivary gland biopsy that was afterwards formalin-fixed and paraffin-embedded. This section stained for La/SS-B with MoAb SW5.8 shows ducts (**arrows**) with freezing artefact (ballooning of the nuclei). The ductal cytoplasm is positive. The acini show small dense immunoreactive nuclei (**arrowhead**) which are compressed to the acinar basement membrane. About 50% of the nuclei of the inflammatory cells in the central part of the section are positive (x 275).

be observed between nSS and pSS patients in the acinar or ductal cell nuclei.

Discussion

We studied the expression of Ro/SS-A and La/SS-B in several healthy and aspecifically acute and chronically inflamed tissues, in the neonatal and adult cardiac conduction systems, and in labial salivary glands of patients suspected of having pSS. Compared to frozen section immunohistochemistry, the major advantage of fixed tissue is the superior morphology obtained in 4 μm paraffin sections. Although fixation might have partly changed the antigenic properties of nuclear proteins such as Ro/SS-A and La/SS-B, this does not seem likely since a multitude of nuclear antigens are nowadays demonstrable by histochemical means using fixed, paraffin-embedded tissue specimens. Moreover, comparison of the streptavidin-biotin-peroxidase technique on paraffin sections with an indirect immunoperoxidase technique on frozen sections yielded similar Ro/SS-A and La/SS-B expression (data not shown).

The present study demonstrates a broad variation in the expression of Ro/SS-A and La/SS-B in human tissues; especially in inflamed tissues the expression appears to be increased. From these ob-

Table IV. Distribution of immunoreactivity levels (low, intermediate or high) for La/SS-B (determined by MoAb 7F6 and SW5.8) in acinar cell nuclei (ACN) and ductal cell nuclei (DCN) of labial salivary gland biopsy specimens from nSS (n = 26) and pSS (n = 15) patients.

	7F6 ACN		7F6 DCN		SW5.8 ACN		SW5.8 DCN	
	nSS	pSS	nSS	pSS	nSS	pSS	nSS	PSS
Low	17	8	18	5	20	7	13	6
Intermediate	6	6	4	3	4	6	12	4
High	3	1	4	7	2	2	1	5
Significance	p = 0.499		p = 0.056		p = 0.136		p = 0.034	

servations we conclude that in 'activated' cells a strong expression of Ro/SS-Aand La/SS-B is induced.

It is known that about 30% of both Ro/SS-A and La/SS-B are localized in the nuclei of cells (15), which is in agreement with the nuclear staining pattern of both proteins found in this study. We were able to demonstrate a weak expression of 60kD Ro/SS-A and La/SS-B in the cytoplasm of salivary gland duct cells, biliary duct cells, hepatocytes and pancreatic duct cells. Since the cytoplasm in comparison to the nucleus constitutes a large part of the cell, it may be expected that the concentration of Ro/SS-Aand La/SS-B in the nucleus would be higher – and consequently immunostaining with MoAbs more intense – than in the cytoplasm. Moreover, in the cytoplasm Ro/SS-Aand La/SS-B are complexed into RNPparticles which might lower their accessibility to the MoAbs. In inflamed tissue specimens the phenomenon of weak immunoreactivity of the cytoplasm compared to the nucleus was more obvious than in the normal tissue specimens. Since we used the streptavidin-biotin-peroxidase technique, cytoplasmic staining could have been induced by endogenous biotin. However, we performed the aforementioned control experiments with an indirect immunoperoxidase technique (data not shown) which confirmed the cytoplasmic 60kD Ro/SS-A and La/SS-B expression.

Nuclear as well as cytoplasmic staining of Ro/SS-Aand La/SS-B has been mentioned in other reports describing fluorescence studies performed with human autoantibodies (18,21,22). However, in none of these reports was formalin-fixed paraffin-embedded tissue used as a substrate, rendering comparison of these and our results not very feasible. Bachmann *et al.* (23) demonstrated the expression of La/SS-B mRNA in the nuclei as well as in the cytoplasm of cells by *in situ* hybridization on fixed and paraffinized tissue sections, which is in accordance with the La/SS-B localization found in our study.

Reliable staining and identification of the nucleoli requires a certain size of these nucleoli, which can be found in neuronal cells of the central nervous system, ganglion cells of the myenteric plexus, large hepatocytes and in hyperplastic, obviously highly active, cells in inflammatory foci. They were found to be negative for both Ro/SS-A and La/SS-B. This finding is in accordance with Veldhoven *et al.* (18), who described the nuclear fluorescence of Hep-2 cells by MoAbs against Ro/SS-A and La/SS-B, in which the nucleoli remained unstained. As mentioned in their paper, formalin fixation might lead to a more diffuse staining pattern, rendering discrimination of the small nucleoli and consequently staining by MoAbs impossible.

The nuclear as well as cytoplasmic expression of Ro/SS-Aand La/SS-B in human cardiac tissue found in our study is in accordance with *in situ* hybridization results (24) in which Y-RNAs were found to be distributed in the nuclei and cytoplasm of human embryonic cardiac tissues and in the cytoplasm of the adult heart. We could not demonstrate La/SS-B, Ro/SS-A 52 kD nor Ro/SS-A 60 kD on the cell membrane of specialized cardiomyocytes of the atrioventricular node or bundle branches in neonatal and adult heart sections. However, tissue sections of the atrioventricular node of a child with CHB were not available.

Ro/SS-A and La/SS-B were expressed in labial salivary gland tissue specimens in both pSS and nSS. However, more pSS patients had high immunoreactivity levels of La/SS-B in ductal cell nuclei than nSS patients. Previous reports, in which other techniques were used, showed an aberrant or increased expression of La/SS-B in acinar cell nuclei in SS salivary gland sections (7,8) as well as in conjunctival epithelial cells of SS patients (25). Because the lymphocytic foci found in labial salivary glands in pSS are often localized around or in the vicinity of the intralobular ducts it is tempting to speculate that increased expression of La/SS-B in ductal cells contributes to the formation of the lymphocytic aggregate.

An additional role could be played by the putative infection of salivary glands with Epstein-Barr virus in SS patients, since it is known that the La/SS-B protein functions as a termination factor for RNA polymerase III, which is used by this virus for its RNA production (11). In theory, an active Epstein-Barr virus infection in salivary glands could lead to high expression of La/SS-B in acinar and ductal cells; the presence of one or more viral proteins in adducts of La/SS-B and viral RNA would suffice to generate an immunogenic particle.

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