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# Anti-La (SS-B) but not anti-$Ro_{52}$ (SS-A) antibodies cross-react with laminin — a role in the pathogenesis of congenital heart block?

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## SUMMARY

Cross-reactions between maternally derived autoantibodies and fetal cardiac antigens have been postulated to play a role in the pathogenesis of congenital heart block (CHB). We have explored the cross-reactivity of autoantibodies to the small ribonuclear autoantigens, La/SS-B and Ro/SS-A, with laminin, the major component of cardiac sarcolemmal membrane using affinity-purified antibodies from patients with Sjögren's syndrome (SS). Anti-La antibodies purified from eight of 10 patients cross-reacted significantly with mouse laminin by ELISA. In contrast, purified antibodies to $Ro_{52}$ from the same 10 patients showed little or no binding to laminin. Laminin inhibited up to 70% binding of anti-La antibodies to La antigen, and La inhibited up to 65% binding of anti-La antibodies to laminin. The cross-reaction was further examined on cryosections of 10 human fetal hearts aged from 8·7 to 14·9 weeks of gestation, two normal adult hearts, and one pathological adult heart with a diagnosis of dilated cardiomyopathy. Anti-$Ro_{52}$ antibodies did not bind to the surface of cardiac cells. However, anti-La antibodies from seven of 10 patients tested bound to the surface of fetal myocytes from hearts aged 9·4 to 14·9 weeks of gestation, and also to the myocytes from the pathological adult heart but not to normal adult hearts. Pre-incubation with La antigen abolished the binding of anti-La antibodies to the surface of adult heart myocytes with dilated cardiomyopathy, and pre-incubation with mouse laminin could partially block this binding. These results suggest that molecular mimicry between laminin and La, but not $Ro_{52}$, may act as a target for specific maternal autoantibodies, and contribute to the pathogenesis of CHB at a critical stage during fetal cardiac development.


**Keywords** congenital heart block autoantibodies La/SS-B cross-reaction laminin

## INTRODUCTION

Congenital heart block (CHB), a potentially fatal and irreversible disease of infants, has been suggested as a consequence of transplacental passage of maternal autoantibodies to the small ribonucleoproteins La/SS-B and Ro/SS-A [1–3]. La (48 kD) is thought to be either a nuclear transcription termination factor [4], and/or a cytoplasmic protein capable of ATP-dependent melting of RNA/DNA hybrids [5]. Ro (52 or 60 kD) is a cytoplasmic antigen, whose precise biological function is unknown. Autoantibodies to Ro and La are found in autoimmune diseases such as primary Sjögren's syndrome (SS) or secondary Sjögren's syndrome accompanied by systemic lupus erythematosus (SLE) [6,7]. Recently, the strongest association with CHB has been described for antibodies to La, either alone or in conjunction with $Ro_{52}$ [2,8,9]. However, only 5% of antibody-positive women are at risk of delivering a child with CHB [10].

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Previous studies by us have demonstrated maternal IgG antibodies bearing anti-La-specific idiotypes on the surface of fetal cardiac myocytes in a single fatal case of CHB. Furthermore, antibodies eluted from the same tissue were shown to react with La *in vitro*, suggesting that either La was aberrantly expressed on the surface of fetal cardiac cells, or that anti-La antibodies cross-reacted with some cardiac sarcolemmal membrane components [11]. Subsequent studies revealed that anti-La antibodies from mothers of children with CHB cross-reacted with cardiac myosin heavy chain ($\beta$ isoform) and laminin, unlike antibodies from mothers of healthy children [12]. These data lent more support to the theory that cross-reactions between maternally derived autoantibodies and fetal cardiac antigens might play a role in the pathogenesis of CHB.

Laminin is a high molecular weight, non-collagenous, structural glycoprotein (900 kD), and is a major component of sarcolemmal membrane of cardiomyocytes and one of the earliest macromolecules expressed in extracellular matrix [13]. Laminin influences cardiac myocytes in several important processes of cell development and in the maintenance of cellular organization. It takes part in cell–cell interactions,

cell attachment, migration and myofibrillogenesis [13–15], and contributes to transmembrane communication between the extracellular matrix and sarcoplasm [15]. As laminin is widely expressed along the surface of cardiomyocytes [16], it may serve as a target for maternally derived autoantibodies crossing the placenta during early fetal heart development. We investigated this by examining the cross-reaction between laminin and anti-La and $Ro_{52}$ antibodies purified from sera of female patients with primary SS. Additionally, we investigated whether the cross-reaction of autoantibodies with laminin *in vitro* resulted in binding of these antibodies to human fetal cardiac tissue.

## PATIENTS AND METHODS

*Sera*

Sera were obtained from 54 patients aged from 29 to 77 years, attending as out-patients at Charing Cross Hospital, London, UK. All patients fulfilled criteria for primary SS [17]. Anti-La and anti-$Ro_{52}$ antibody levels in each serum were measured by indirect ELISA and compared with the level of antibodies in sera collected from 45 healthy unrelated individuals. Sera from 10 patients with high levels of both anti-La and anti-$Ro_{52}$ antibodies were selected for the purification of antibodies. None of these patients had previous histories of CHB-affected children.

*Affinity purification and biotinylation of antibodies*

IgG anti-La and anti-$Ro_{52}$ antibodies were purified from the sera of patients by affinity chromatography on purified rabbit La cross-linked to immobilized antibody [18] or human placental $Ro_{52}$ coupled directly to cyanogen bromide (CNBr)-activated Sepharose (Pharmacia, Uppsala, Sweden). The IgG concentration of antibodies was quantified by inhibition ELISA as previously described [19] and screened by direct ELISA for reactivity with La purified from rabbit thymus extract (Pel-Freez Biologicals, Rogers, AK) as previously described [20] or recombinant human $Ro_{52}$ protein, a gift from Professor W. J. van Venrooij (University of Nijmegen, The Netherlands). Aliquots of purified antibodies were conjugated to NHS-Biotin (Pierce Chemical Co, Rockford, IL), and stored at −70°C [12].

*ELISA*

ELISAs were performed as previously described [12]. Briefly, rabbit La (10 μg/ml) or $rRo_{52}$ (0·5 μg/ml) in 10 mM Tris, 1 mM EDTA pH 9, was coated onto ELISA plates (Dynatech Labs Inc., Chantilly, VA) overnight at 4°C. Laminin from Engelbreth–Holm–Swarm (EHS) mouse sarcoma (Sigma, Poole, UK) was coated at 10 μg/ml in PBS pH 7·2. Serial four-fold dilutions of biotinylated antibodies binding to these antigens were detected using an avidin–alkaline phosphatase conjugate (Sigma), and developed with phosphatase substrate (Sigma). Rabbit polyclonal anti-mouse laminin antibodies (Sigma) were used as a positive control in the laminin ELISA and detected by goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma). As a negative control biotin-conjugated pooled human IgG (Sigma) was tested at equivalent concentrations to patient antibodies. The optical density was measured by a Biotech microplate spectrophotometer at 405 nm. The results were standardized by reference to the internal positive controls in each ELISA plate.

*SDS–PAGE and Western blotting*

La and $Ro_{52}$ proteins in a cytoplasmic extract of Molt4 cells were separated by electrophoresis under reducing conditions in 15% polyacrylamide–SDS gels according to a previously published method [21]. Mouse EHS laminin was electrophoresed under reducing conditions in 3–10% polyacrylamide gradient gels according to the method of Laemmli [22]. Proteins were electrotransferred to nitrocellulose and probed as follows: on Molt4 immunoblots proteins were detected with purified human anti-La and anti-$Ro_{52}$ and rabbit anti-laminin antibodies (non-biotin conjugated) and developed with goat anti-human IgG (γ chain-specific), or goat anti-rabbit IgG horseradish peroxidase conjugate (HRP) (ICN Biomedicals Inc., High Wycombe, UK), and on laminin immunoblots polypeptides were detected with purified mouse monoclonal or human polyclonal anti-La antibodies conjugated to biotin and rabbit anti-laminin antibodies (non-biotin-conjugated). Biotin-conjugated antibodies were detected by streptavidin–HRP (Serotec, Oxford, UK).

*Cardiac tissues and immunofluorescence*

Human fetal heart tissues, aged from 8·7 to 14·9 weeks of gestation, were obtained from the Medical Research Council Tissue Bank with approval from the Ethical Committee of Charing Cross Hospital, London, UK. Post-mortem normal human adult heart tissues were provided by Dr D. V. Parums (Department of Histopathology, Hammersmith Hospital, London, UK) and Dr D. Woodrow (Department of Histopathology, Charing Cross Hospital). Heart tissue with a diagnosis of dilated cardiomyopathy was supplied by Dr M. Rose (Harefield Hospital, UK). Tissues were snap-frozen and stored at −70°C. Sequential cryosections (6 μm) were cut at −20°C and fixed in acetone/methanol (1 : 1 v/v) for 10 min at −20°C. All procedures for immunofluorescent staining were carried out at room temperature. Cryosections were blocked with 20% normal goat serum for 30 min, then washed three times with PBS. For immunofluorescent detection using biotin-conjugated antibodies, sections were blocked once more using an avidin/biotin blocking kit (Vector Labs, Burlingame, CA). After blocking, tissues were incubated either with 30 μl of buffer alone as a conjugate control, or with 30 μl purified, biotin-conjugated anti-La or anti-$Ro_{52}$ antibodies (50 μg IgG/ml) for 30 min. Two affinity-purified mouse anti-La MoAbs (SW1 and SW5) generated in this Institute were also conjugated to biotin and applied to fetal cardiac sections (4 μg IgG/ml) [23]. Rabbit anti-laminin antibodies (1 : 200 dilution) were used as positive controls. After washing, bound antibodies were sequentially detected with FITC-conjugated streptavidin (Amersham, Aylesbury, UK) or FITC-conjugated goat anti-rabbit IgG (Sigma). Specific immunofluorescent staining of heart tissue was identified under ultraviolet (UV) light with a Leitz Dialux 22EB microscope using a water immersion lens (magnification ×100), and photographed (final magnification × 1000). Haematoxylin and eosin (H&E) staining was routinely performed for each heart tissue and examined by light microscopy.

*Inhibition assay*

The fluid-phase inhibition experiments were performed as previously described either in ELISA or on cardiac tissue sections [12]. A single dilution of affinity-purified and biotin-

conjugated anti-La antibodies was pre-incubated with either buffer alone or with increasing concentrations of La (1, 10, 100 μg/ml) or laminin (1, 10, 100, 800 μg/ml) as inhibitor. Mixed samples were then transferred to La or laminin-coated ELISA plates or directly onto cardiac sections. Binding to La or laminin by ELISA in buffer alone was expressed as 100%, and inhibition by different antigens was calculated by reference to this. Inhibition of binding to cardiac tissues was photographed and assessed visually.

## RESULTS

*Binding of anti-La and anti-$Ro_{52}$ antibodies to laminin*

Anti-La and anti-$Ro_{52}$ antibodies from 10 patients with SS (nos 1–10) were screened in duplicate over four four-fold dilutions for cross-reactivity with laminin by ELISA. Anti-La antibodies bound to laminin in a dose-related manner, and the proportion which bound was much greater with anti-La than with anti-$Ro_{52}$ antibodies. To compare the binding activity to laminin directly between anti-La and anti $Ro_{52}$, antibodies were first diluted to give an OD of 0·600 on La-coated plates or an OD of 1·000 on $Ro_{52}$-coated plates. All antibodies were screened for cross-reactivity with laminin in the same ELISA plate. Eight of 10 anti-$Ro_{52}$ antibodies showed no binding to laminin, while two reacted very weakly. In contrast, eight of 10 anti-La antibodies showed binding to laminin, and six of these reacted strongly (patients 1, 3, 4, 6, 7 and 10) (Fig. 1a). Although normal human sera contain low levels of IgG antibodies which bind to laminin (Horsfall, unpublished observations), the binding of pooled normal human IgG to La and laminin was comparable to that of anti-$Ro_{52}$ antibodies pooled from all 10 patients (Fig. 1b,c).

*Inhibition ELISA*

Inhibition experiments were performed on anti-La antibodies from six patients which showed the strongest reactivity with laminin (nos 1, 3, 4, 6, 7 and 10) using either La or laminin as inhibitor. Pre-incubation of anti-La antibodies with La in the fluid phase inhibited the binding of all six antibodies to solid-phase laminin in a dose-dependent manner. At the highest dose of soluble La antigen (100 μg/ml), the inhibition of binding to laminin of six anti-La antibodies ranged from 60% to 91%, and to La ranged from 90% to 97%. In contrast, pre-incubation of soluble laminin with anti-La antibodies resulted in less inhibition of binding to solid phase La. At the same dose of laminin (100 μg/ml) binding to La of six anti-La antibodies was only partially inhibited. The mol. wt of La is approximately 48 kD and that of laminin is 900 kD, which means that on a weight basis a 20-fold excess of laminin over La would be required to give equimolar concentrations. Laminin is supplied commercially as a 1 mg/ml solution, and therefore the highest dose of laminin available for inhibition was 800 μg/ml. At this dose we achieved inhibition of 85–97% for binding to laminin, and 71–89% for binding to La (Table 1).

*Western blotting*

In Western blots of Molt4 extract, a strong specific reaction of anti-La and anti-$Ro_{52}$ antibodies was observed with their respective antigens, resulting in clear, narrow bands at 48 kD (La) or 52 kD (Ro), while rabbit anti-mouse laminin antibodies did not bind (Fig. 2, left panel). We also looked for the presence of La in the mouse EHS laminin preparation by Western blotting. Two bands were detected by polyclonal rabbit anti-laminin antibodies at approximately 400 kD (A chain) and approximately 200 kD (B1 and B2 chains). Some degradation bands were also detected by anti-laminin antibodies. Anti-La antibodies (patients 2 and 4, SW1 and SW5) did not recognize any bands on Western blots, which confirmed the lack of contamination of the laminin with La, and also suggested the cross-reaction between anti-La and laminin was conformationally dependent (Fig. 2, right panel).

*Immunohistochemistry*

Histologically, fetal cardiac cells were small compared with adult myocardial cells, and surface laminin expression revealed by rabbit anti-laminin antibodies showed a discontinuous patchy pattern (Fig. 3a). Anti-$Ro_{52}$ and anti-La antibodies purified from 10 patients and anti-La MoAbs SW1 and SW5 were used for immunofluorescent staining of tissue sections from 10 fetal hearts aged from 8·7 to 14·9 weeks of gestation.

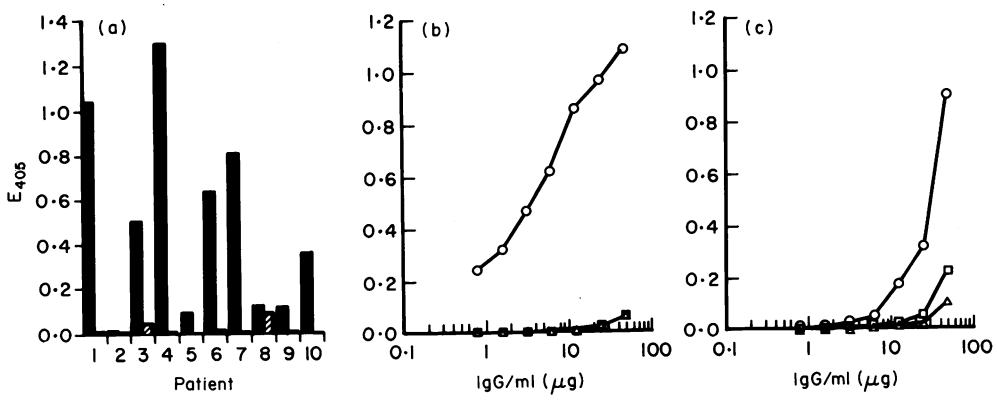


**Fig. 1.** Biotin-conjugated anti-La and anti-$Ro_{52}$ antibodies purified from 10 patients with primary Sjögren's syndrome (SS) were screened for binding to La and mouse Engelbreth–Holm–Swarm (EHS) laminin by ELISA. (a) Binding to laminin. Antibody dilutions were selected to give an $E_{405}$ of 0·600 in La ELISA (■) and 1·000 in $Ro_{52}$ ELISA (▨). (b,c) Binding to La and laminin, respectively, of anti-La (○) and anti-$Ro_{52}$ (△) antibodies pooled from all 10 patients and of normal IgG (□) as a function of IgG concentration.

**Table 1.** Inhibition ELISA

| Inhibitor | On La-coated plate | | | | | On laminin-coated plate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | None | La (100 μg/ml) | | Laminin (800 μg/ml) | | None | La (100 μg/ml) | | Laminin (800 μg/ml) | |
| Patient | $E_{405}$ | $E_{405}$ | Inhibition, % | $E_{405}$ | Inhibition, % | $E_{405}$ | $E_{405}$ | Inhibition, % | $E_{405}$ | Inhibition, % |
| 1 | 0·576 | 0·059 | 90 | 0·124 | 78 | 0·784 | 0·317 | 60 | 0·026 | 97 |
| 3 | 0·450 | 0·045 | 90 | 0·129 | 71 | 0·264 | 0·048 | 82 | 0·019 | 93 |
| 4 | 0·455 | 0·035 | 92 | 0·059 | 87 | 0·293 | 0·087 | 70 | 0·029 | 90 |
| 6 | 0·506 | 0·034 | 93 | 0·109 | 78 | 0·365 | 0·092 | 75 | 0·054 | 85 |
| 7 | 0·401 | 0·040 | 90 | 0·045 | 89 | 0·296 | 0·028 | 91 | 0·017 | 94 |
| 10 | 0·550 | 0·014 | 97 | 0·089 | 84 | 0·342 | 0·085 | 75 | 0·037 | 89 |

Binding of anti-La antibodies to La or laminin in the absence of inhibitor was expressed as 100%, and inhibition by La or laminin was calculated by reference to this.

All 10 anti-$Ro_{52}$ antibodies gave weak perinuclear and cytoplasmic staining on eight fetal cardiac sections aged between 10·3 and 14·9 weeks of gestation (Fig. 3b), but were negative on sections from fetal hearts of 8·7 and 9·4 weeks of gestation. Surface staining was not seen with any anti-$Ro_{52}$ antibodies. All 10 anti-La antibodies gave nuclear and cytoplasmic staining. However, two anti-La antibodies (patients 5 and 6) gave strong nuclear staining and weaker cytoplasmic staining, but showed no evidence of surface staining (Fig. 3c). In contrast to anti-$Ro_{52}$ antibodies, anti-La antibodies from seven patients showed additional surface staining (Fig. 3d), while those from patient 1 showed weak surface staining on different fetal cardiac tissue aged from 9·4 to 14·1 weeks of gestation (Table 2). No surface staining was seen with any of the 10 anti-La antibodies on fetal cardiac tissue of 8·7 weeks gestation. MoAb SW5 stained the surface of fetal myocytes from 9·4 to 12·8 weeks of gestation, with peak intensity of staining at 10·3–12·6 weeks of gestation. At 14·8 and 14·9 weeks of gestation SW5 showed no surface staining (Table 2). Double staining on a fetal cardiac section (10·3 weeks gestational age) with anti-laminin detected with FITC-conjugated anti-rabbit IgG (Fig. 3e), and anti-La antibodies (patient 4) detected with streptavidin Texas red showed similar surface staining with both antibodies (Fig. 3f).

Two anti-La antibodies (patients 4 and 9) which showed positive surface staining on fetal cardiac sections and two (patients 1 and 6) showing little or no surface staining were selected for immunofluorescence on adult cardiac tissue. Myocytes from diseased heart were irregular in size, with obvious hypertrophy. No marked myocyte necrosis nor aggregation of inflammatory cells was seen. Myocytes from normal adult heart stained with anti-laminin showed a uniform staining pattern along the entire surface, whereas those from diseased heart showed increased expression of laminin and thickness of the

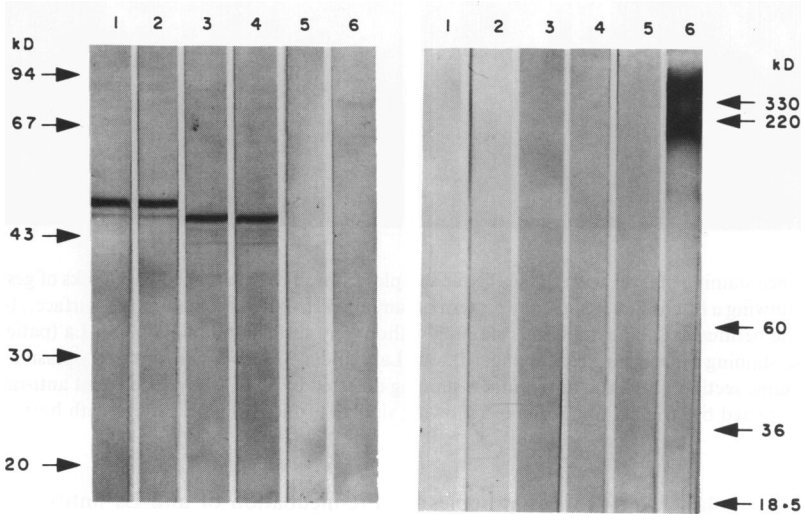


**Fig. 2.** Western blotting of anti-La and anti-$Ro_{52}$ antibodies. Left panel: cytoplasmic extract from Molt4 cells. Lane 1, anti-$Ro_{52}$ (patient 2); lane 2, anti-$Ro_{52}$ (patient 4); lane 3, anti-La (patient 2); lane 4, anti-La (patient 4); lane 5, anti-human IgG–horseradish peroxidase (HRP) conjugate; lane 6, rabbit anti-laminin. Right panel: mouse Engelbreth–Holm–Swarm (EHS) laminin. Lane 1, monoclonal anti-La (SW1); lane 2, monoclonal anti-La (SW5); lane 3, anti-La (patient 2); lane 4, anti-La (patient 4); lane 5, anti-rabbit IgG–HRP conjugate; lane 6, rabbit anti-laminin. Arrows indicate standard molecular weight markers.

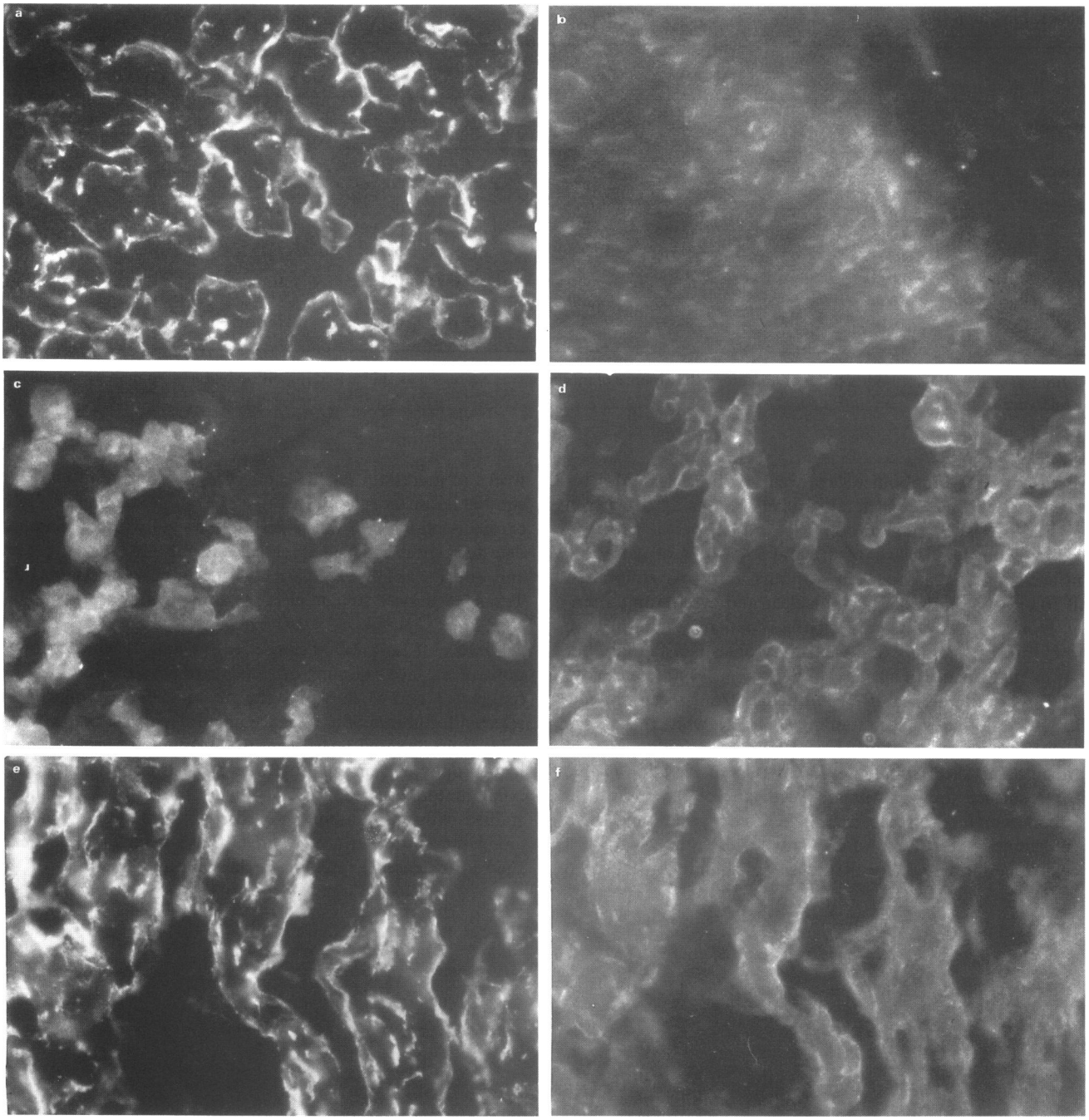

**Fig. 3.** Immunofluorescence staining of fetal heart tissue. Tissue samples from a fetal heart aged 10·3 weeks of gestation were stained by (a) rabbit anti-laminin showing a discontinuous, patchy pattern of laminin distributed around the cell surface; (b) anti-$Ro_{52}$ (patient 9) showing discrete punctate perinuclear and cytoplasmic staining without any surface staining; (c) anti-La (patient 6) showing distinct nuclear and cytoplasmic staining but no surface staining; (d) anti-La (patient 9) showing nuclear, cytoplasmic and surface staining. Double staining on the same section: (e) rabbit anti-laminin staining detected by FITC-conjugated goat anti-rabbit IgG, and (f) anti-La (patient 4) staining detected by streptavidin Texas red showing similar surface staining pattern with both antibodies.

sarcolemmal membrane (Fig. 4a). Anti-$Ro_{52}$ antibodies (patients 1, 4, 6 and 9) did not stain the surface of myocytes in sections of either normal or diseased adult heart (Fig. 4b). However, the section from the diseased tissue showed cell surface staining by anti-La antibodies from patients 4 (Fig. 4c) and 9, none with anti-La antibodies from patients 1 and 6. Pre-incubation of anti-La antibodies (patients 4 and 9) with soluble La (100 $\mu$g/ml) abolished the surface staining (Fig. 4d), and pre-incubation of anti-La antibodies with soluble laminin (100 $\mu$g/ml) partially blocked this staining (Fig. 4e). Similar inhibition was observed using anti-La antibodies from patient 2 on fetal cardiac sections of 14·9 weeks gestation (data not

**Table 2.** Surface staining of fetal cardiac cells by anti-La antibodies

| Heart no. | Age, weeks | Purified anti-La antibodies from patients | | | | | | | | | | MoAbs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | SW1 | SW5 |
| 10869 | 8·7 | − | − | − | − | − | − | − | − | − | − | − | − |
| 10868 | 9·4 | + | +++ | ++ | +++ | − | − | − | − | + | +++ | − | + |
| 10939 | 10·3 | + | + | − | ++ | − | − | ++ | ++ | +++ | +++ | − | ++ |
| 11053 | 11·2 | − | ++ | + | − | − | − | + | − | − | ++ | − | ++ |
| 10845 | 12·6 | − | +++ | ++ | − | − | − | +++ | + | − | ++ | − | ++ |
| 10855 | 12·8 | − | + | +++ | − | − | − | +++ | +/− | − | − | + | +/− |
| 10916 | 13·1 | − | − | − | + | − | − | − | − | ++ | + | − | − |
| 10885 | 14·1 | +/− | ++ | +/− | ++ | − | − | ++ | − | +++ | ++ | − | + |
| 10924 | 14·8 | − | − | − | − | − | − | − | − | − | − | + | − |
| 10938 | 14·9 | − | +++ | + | − | − | − | + | − | − | + | +/− | − |

Fluorescent staining of the surface of cardiomyocytes from each fetal heart tissue by rabbit anti-laminin antibodies was used as a positive control. The intensity of fluorescence obtained with FITC-conjugated anti-rabbit antibodies alone was used as a negative control. Positive surface staining obtained with anti-La antibodies on each section covering > 80% of myocytes was considered as +++, covering 50–80% of myocytes was considered as ++, covering 20–50% of myocytes was considered as +, < 20% was considered as +/−. MoAbs, Mouse monoclonal anti-rabbit La antibodies.

shown). No surface staining was observed in sections of normal adult heart incubated with anti-La antibodies (Fig. 4f).

## DISCUSSION

Our data have demonstrated a cross-reaction between laminin and anti-La antibodies purified from the sera of 10 patients with primary SS. While six anti-La antibodies cross-reacted significantly with mouse laminin by ELISA, and seven bound to the sarcolemmal membrane of human fetal cardiomyocytes aged from 9·4 to 14·9 weeks of gestation, only four anti-La antibodies (patients 3, 4, 7 and 10) cross-reacted with mouse laminin by ELISA and also bound to normal human fetal but not adult cardiac sections. These cross-reactions could also be partially inhibited with both La and laminin. Anti-$Ro_{52}$ antibodies purified at the same time showed no cross-reactivity with laminin. Although CHB was associated with anti-Ro antibodies in earlier studies, more recent data have suggested that a stronger association exists with antibodies to La or La plus $Ro_{52}$ [2,8,9]. The strong association of anti-$Ro_{52}$ antibodies with CHB may well reflect the frequent occurrence of anti-La and anti-$Ro_{52}$ antibodies together in patients with primary SS. Our data suggest that of the two, anti-La antibodies may be pathogenic by virtue of the cross-reaction with fetal cardiac laminin. If anti-$Ro_{52}$ antibodies are pathogenic then this involves another as yet unknown mechanism.

Anti-La antibodies recognize multiple epitopes on the La polypeptide [24–26]. La shares 17 matches of four or more consecutive amino acids with the laminin B1 chain, including a match of six consecutive amino acids (EAKLRA) between residues 202–207 of the La protein and residues 1467–1472 of the laminin B1 chain [12]. These short linear sequences shared between La and laminin B1 chain may contribute to the cross-reactivity of anti-La antibodies with laminin. Laminin is composed of three different polypeptides interacting to form a triple $\alpha$-helical coiled-coil structure [13–15]. The $\alpha$-helical coiled-coil molecules have been implicated in cardiac autoimmunity, as cytotoxic anti-streptococcal antibodies have been shown not only to react with streptococcal M protein but also to a group of $\alpha$-helical coiled-coil molecules, such as Coxsackievirus capsid proteins, cardiac myosin and laminin [27,28]. The La protein also contains $\alpha$-helical regions which may be recognized by a subset of anti-La antibodies, thus enabling cross-reactivity with other coiled-coil proteins. If such a structure is involved in the cross-reaction of anti-La antibodies with laminin, the epitopes on the laminin molecule recognized by anti-La antibodies would be conformationally dependent. This is supported by our data, which showed that anti-La antibodies recognized native laminin by ELISA and bound to cardiac tissue sections, but did not recognize denatured laminin by Western blotting.

As laminin is a large protein, each molecule could have several epitopes recognized by cross-reactive anti-La antibodies. Different anti-La antibodies might cross-react with different epitopes expressed on human cardiac laminin or mouse EHS laminin. Patient 6 is of interest, as antibodies were purified from this patient during a pregnancy which resulted in the birth of a healthy child. These antibodies cross-reacted with mouse laminin by ELISA, but did not bind to human cardiac laminin, while others, such as those purified from patients 2 and 9, showed more reactivity with human cardiac laminin than with murine laminin. Compared with mouse EHS tumour laminin, human cardiac laminin lacks the 400-kD A chain, but has a 300-kD polypeptide which is not antigenically related to the EHS laminin A or B chains [29–31]. There are other differences between human cardiac laminin and mouse EHS laminin [15]. The heterogeneity among laminin molecules may explain why pre-incubation with soluble mouse EHS laminin could not totally block the binding of anti-La antibodies to human cardiac laminin, and the discordance between the two assays.

Our observation that anti-La antibodies bound to the surface of myocytes in normal fetal but not adult hearts is compatible with reports that laminin molecules undergo conformational structural changes during development [32,33]. The onset of CHB has been documented as early as week 16

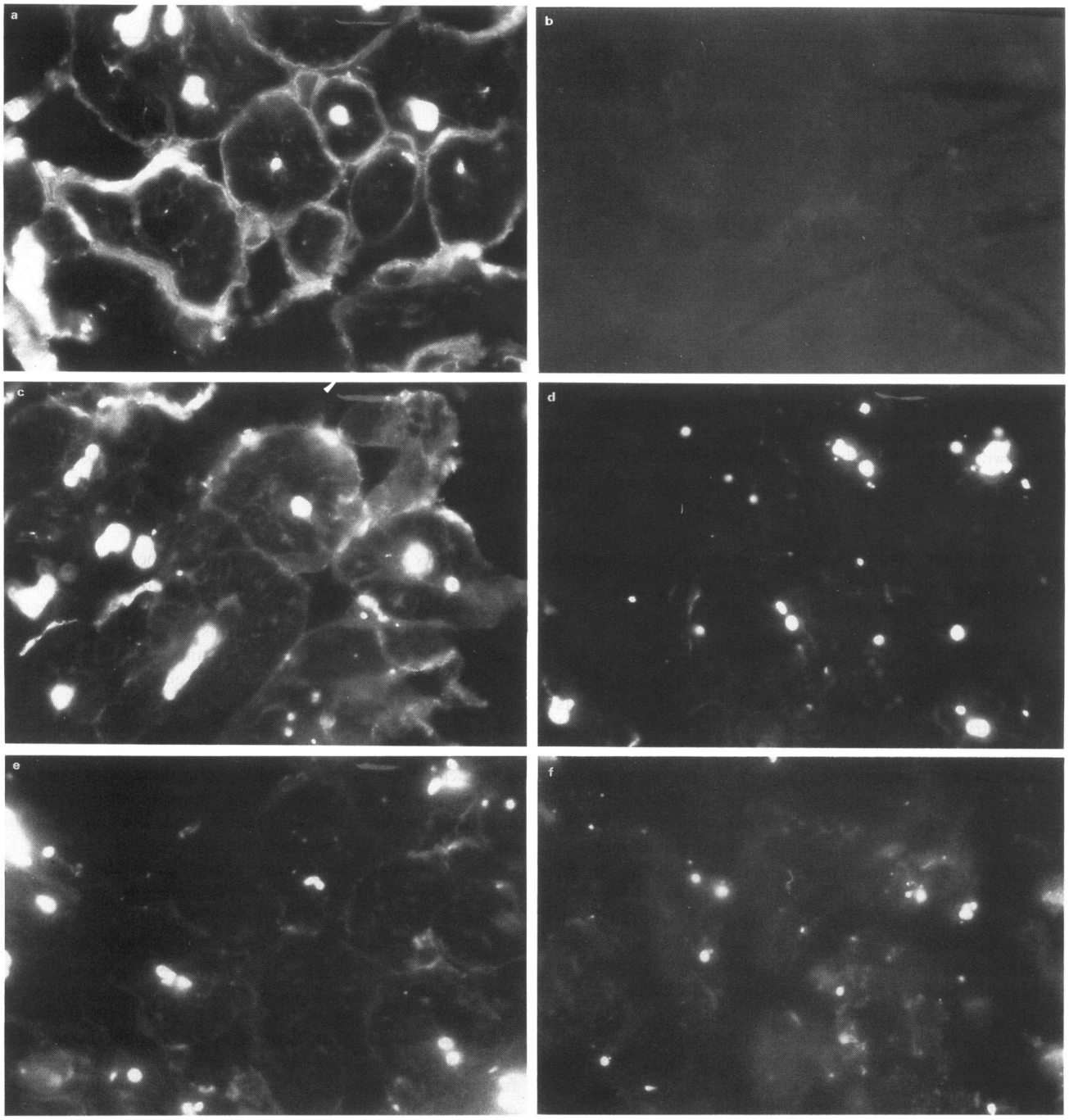


**Fig. 4.** Immunofluorescence staining of adult cardiac tissues: (a) rabbit anti-laminin staining of a section from diseased heart (dilated cardiomyopathy) showing laminin distributed along the entire cell surface; (b) anti-$Ro_{52}$ (patient 4) showing no staining of diseased heart tissue; (c) anti-La (patient 4) showing cytoplasmic and surface staining of diseased heart tissue; (d) pre-incubation with soluble La (100 $\mu$g/ml) abolished the staining of anti-La (patient 4) on diseased heart tissue; (e) pre-incubation of anti-La (patient 4) with soluble mouse laminin (100 $\mu$g/ml) partially blocked the surface staining of diseased heart tissue; (f) anti-La (patient 4) showing discrete cytoplasmic staining of normal adult heart tissue.

of gestation, implying that the immune events which lead to inflammation and cardiac damage precede this time [3]. With laminin undergoing structural modulation during cardiac development, maternally derived cross-reactive autoantibodies crossing the placenta during the first trimester could recognize fetal cardiac laminin and initiate tissue injury, while having no effect on maternal adult heart. Antibody deposition on the surface of rabbit fetal heart cells has been shown to inhibit cardiac repolarization, leading to heart block [34]. The distribution of laminin in the basement membrane of cardiomyocytes differs between the embryo, the neonate and the adult. During embryonic development, laminin is located in

punctate patches on the surface of the plasma membrane, in neonates its localization is more extensive with only few gaps, whereas in the adult heart laminin is distributed along the entire basement membrane [35]. The fetal hearts used in our study were aged between 8·7 and 14·9 weeks of gestation, and staining with polyclonal anti-laminin antibodies revealed discontinuous surface expression of laminin. The development of heart from a single tube into a four-chambered pump involves continuous remodelling of cardiac structure, and is accompanied by expression of special antigens in the developing embryonic heart [36]. It has been reported that the B chain subunits of laminin are synthesized earlier than the A chain [37], and that immunoreactivity of laminin in the basement membrane changes from developing to adult cardiac tissue [32].

We have demonstrated the cross-reactivity of anti-La antibodies with laminin *in vitro* and the binding of these autoantibodies to fetal cardiac sections, implying that such cross-reactivity could occur *in vivo*. The clinical significance of this finding must be addressed in the light of what is known about CHB. Many women may be asymptomatic, and a few have no detectable autoantibodies at the time of a CHB pregnancy but become antibody-positive at a later date. Absorption of low levels of cross-reactive antibodies by a fetal heart during the first trimester may well be below the limits of detection in maternal sera. A recent report shows that the placenta as well as neonatal heart and kidney are particularly rich in laminin B1- and B2-chains [31]. Absorption of cross-reactive anti-La antibodies by laminin in placenta may afford some protection to the fetus, and emphasizes the functional role of the placenta as well as antibody status as risk factors for CHB.

We propose the hypothesis that placental transfer of maternal cross-reactive anti-La antibodies during a critical early stage of fetal cardiac development may provide a mechanism for the subsequent development of CHB. An important future experiment would be to elute antibodies from fetal heart in a fatal case of CHB and to examine their specificity for La and Ro and cross-reactivity with human laminin. While not conclusively proving pathogenicity, this would address our hypothesis, and identify which autoantibodies are truly risk factors for CHB.

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