

## Enhanced membrane expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens by human keratinocytes induced by TNF $\alpha$

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### Abstract

**Objective**—To investigate the membrane expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens in human keratinocytes under the influence of an important mediator of inflammation, TNF $\alpha$ .

**Methods**—Keratinocytes, isolated from human skins obtained at circumcision and identified using monoclonal antibodies, were treated with tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and incubated with antibodies to 52 kDa Ro(SS-A) isolated and purified from patients with systemic lupus erythematosus or Sjögren's syndrome, with mouse monoclonal antibody to La(SS-B), and (as controls) with sera from normal healthy blood donors and a mouse monoclonal antibody to U1RNP 68 kDa. Membrane expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens was detected using cyto enzyme linked immunosorbent assays (ELISAs), laser scanning microscopy, and indirect immunofluorescence.

**Results**—After the incubation with TNF $\alpha$ , cyto ELISA revealed a significantly increased membrane binding of 52 kDa Ro(SS-A) antibodies, with a maximum after two hours, followed by enhanced 52 kDa Ro(SS-A) expression during the subsequent 24 hours. The La(SS-B) antigen was expressed rapidly after TNF $\alpha$  treatment (within one hour), with a fast decrease to the preincubation value within three hours. Indirect immunofluorescence with fixed normal human keratinocytes confirmed increased 52 kDa Ro(SS-A) and La(SS-B) antigen expression after the incubation with TNF $\alpha$ .

**Conclusions**—TNF $\alpha$  mediates 52 kDa Ro(SS-A) and La(SS-B) autoantigen surface expression on human keratinocytes, and may be an important factor both in antibody induction and in the initiation of immunopathogenic processes which occur after antibody binding in autoimmune dermatitis.

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The diagnostic value of antibodies to the Ro(SS-A) and La(SS-B) antigens is well documented for Sjögren's syndrome, different varieties of lupus erythematosus, and congenital heart block. Moreover, autoantibodies

to Ro(SS-A) are significantly associated with photosensitivity, dermatitis, vasculitis, pulmonary involvement, renal disease, and lymphopenia, suggesting their immunopathogenic role in patients with systemic lupus erythematosus, in addition to Sjögren's patients.<sup>1-5</sup> However, the precise pathogenic mechanisms leading to specific tissue involvements and inflammatory manifestations are unclear. There is little information about the interaction of these antigens with their autoantibodies. Ultraviolet light,<sup>6-10</sup> oestradiol,<sup>8, 11</sup> and adenovirus infection<sup>12, 13</sup> have been shown to enhance anti-Ro(SS-A) and anti-La(SS-B) binding on the surface of keratinocytes, though in unstimulated cells the corresponding antigens are found only within the cell. Furthermore, it has been demonstrated that Ro(SS-A), La(SS-B), and other autoantigens exist in two different bleb types of apoptotic keratinocytes after ultraviolet B irradiation.<sup>10</sup> The mediators involved in the upregulation of anti-Ro(SS-A) cell surface binding of keratinocytes induced by ultraviolet B are not known. Because ultraviolet radiation induces release of cytokines (tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukins (IL) 1, 6, 8 and 10) by human keratinocytes, and TNF $\alpha$  induces other antigens such as proteinase 3 on the cell surface of human endothelial cells,<sup>14</sup> we investigated the membrane expression of the 52 kDa Ro(SS-A) antigen and La(SS-B) protein in human keratinocytes under the influence of TNF $\alpha$ .

### Patients and methods

#### SERUM SAMPLES

High titre anti-Ro(SS-A) positive sera were obtained from three mothers with systemic lupus erythematosus (SLE) and secondary Sjögren's syndrome who gave birth to children suffering from congenital heart block, and from 12 additional anti-Ro(SS-A) positive SLE patients, for the purification of anti-52 kDa Ro(SS-A) antibody. In addition, we tested two monospecific anti-52 kDa Ro(SS-A) positive sera obtained from patients with primary Sjögren's syndrome (designated pSS1 and pSS2), and a mouse monoclonal antibody to La(SS-B) (a gift from Dr M Bachmann, Mainz, Germany). A serum pool from 150 normal healthy blood donors (NHS) and one mouse monoclonal antibody to U1 ribonucleoprotein (U1RNP) 68 kDa (Progen Biotechnik, Heidelberg, Germany) served as controls.

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## PURIFICATION OF ANTI-52 KDa RO(SS-A)

## ANTIBODIES

Recombinant 52 kDa Ro(SS-A) antigen<sup>15</sup> was dialysed overnight at 4°C against coupling buffer (0.1 mol/l sodium bicarbonate, 0.5 mol/l sodium chloride, pH 8.3), and coupled to cyanogen bromide activated Sepharose 4B (2 mg antigen/ml gel; Pharmacia, Uppsala, Sweden), prepared according to the manufacturer's instructions. Remaining binding sites were blocked with 1.0 mol/l ethanolamine for 16 hours at 4°C and 5 ml of antigen linked Sepharose packed in a column. Before use, the columns were washed with three cycles of alternating coupling buffers and 0.1 mol/l acetate buffer, pH 4, containing 0.5 mol/l sodium chloride, followed by coupling buffer and finally phosphate buffered saline (PBS), pH 7.4. Anti-52 kDa antibodies were purified using the 52 kDa Ro(SS-A) column and 30 ml mixed sera (10 ml/h). Columns were washed with 20 column volumes of PBS before elution with 0.1 mol/l glycine/hydrochloric acid, pH 2.5. Bound immunoglobulin was eluted and neutralised in Tris buffer, dialysed against PBS at 4°C, and concentrated to approximately 0.5 mg/ml.

## ANTIBODY TESTING

The specificity of sera used was determined by a routine indirect immunofluorescence technique for antinuclear antibodies, counter immunoelectrophoresis, immunoblotting, and enzyme linked immunoassays as described previously.<sup>16</sup> Specificity of the purified antibodies to 52 kDa Ro(SS-A) (Progen Biotechnik), recombinant 60 kDa Ro(SS-A), La(SS-B), and U1RNP-A, -C, 68 kDa was also determined by enzyme immunoassay as described previously.<sup>16</sup> Immunoblot analysis was performed with antinuclear antibody/anti-mitochondrial antibody blot strips detecting antibodies to the antigens 52 and 60 kDa Ro(SS-A), La(SS-B), RNP, Sm, Jo, Scl-70, Histon-1, PM-Scl, and anticentromere and anti-mitochondrial antibodies (AID, Freiburg, Germany). The 52 kDa Ro(SS-A) antibody containing probes were tested in an inhibition assay using different concentrations of the 52 kDa recombinant antigen (10, 1, 0.1, 0.01  $\mu$ g/ml<sup>15</sup>). Antibody reactivities to a different 52 kDa Ro(SS-A) antigen<sup>17</sup> (Progen Biotechnik) were also measured.

## HUMAN KERATINOCYTE CULTURES

Human foreskins removed during routine circumcision of newborn or juvenile male infants were dissected into small pieces. After rinsing in sterile PBS, the subcutaneous tissue was eliminated mechanically and tissue samples were floated on 0.25% trypsin at 4°C overnight (12–18 h). The epidermis was separated from the dermis using fine curved forceps. A single cell suspension was prepared from epidermal fragments by repeated vigorous pipetting and sieving through gauze. Resulting cells were washed in serum containing culture medium (trypsin inhibition)

by centrifugation, counted for total and viable cells, and plated at  $1-2 \times 10^4$  cells/cm<sup>2</sup> with 3T3 fibroblasts growth arrested either by irradiation or treatment with mitomycin C, at 37°C in medium. In cultures grown to sub-confluency, 3T3 feeder cells and any human dermal fibroblasts were selectively removed by ethylenediamine (EDTA).<sup>18, 19</sup> The resulting keratinocytes were trypsinated and after a subcultivation procedure the cell suspension was plated in low Ca<sup>2+</sup> serum free medium containing epidermal growth factor and bovine pituitary extract (Gibco, Germany). Monoclonal antibodies were used to identify human keratinocytes or to exclude contamination with mesenchymal cells (anti-pan cytokeratin-fluorescein isothiocyanate (FITC) (Sigma Chemicals, Germany): anti-vimentin, HMB 45, anti-CD1a (Dako, Germany), and S-100, (Medac, Germany)). Cells of passages 3–5 were used for all experiments. Cell viability was determined by acridine orange/ethidium bromide (AO/EB) staining.<sup>11</sup>

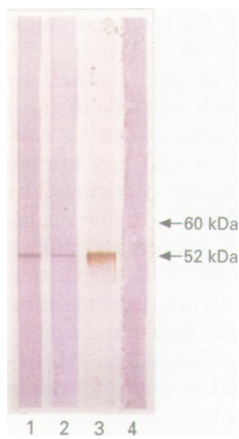
## INDIRECT IMMUNOFLUORESCENCE

Aliquots of 1 ml of second passage normal human keratinocytes (NHK) (40 000 cells) were plated in each well of Lab-Tek chamber slides (Miles Lab, Naperville) and cultured for four days in RPMI, then 150  $\mu$ l of TNF $\alpha$  (4 ng/ml) was added to the wells. Partly confluent NHK were also treated with trypsin/EDTA, transferred onto amino-3-propyltriethoxysilane (APES) coated slides to prevent loss of antigenicity, and incubated in a moist chamber at room temperature for 30 minutes. After an incubation with TNF $\alpha$  for one, two, four, six, or 24 hours and subsequent blocking of Fc receptors with AB serum (Biogenes GmbH, Berlin, Germany), each well was washed with PBS and the slides incubated with antibody solutions or antisera at 4°C. After further washing three times with PBS, pH 7.4 (4°C), specimens were incubated with AB serum to block Fc receptors, followed by incubation with 1 mg/l of FITC conjugated antihuman or antimouse IgG (to detect anti-mLa(SS-B) and anti-m68 kDa U1RNP, respectively) for one hour at 4°C. After a further washing step, the specimens were mounted in Mowiol 1% (Hoechst, Germany) and examined using light and laser scanning microscopy (LSM 10; Zeiss, Germany).

## CYTO ELISAS

These were performed with unfixed cytokine treated NHK according to the method described by Frampton *et al.*,<sup>20</sup> with minor modifications. For determination of specific antibody binding, experiments were performed with NHK preincubated with heat aggregated human IgG (Biogenes GmbH) to block Fc receptors, and with immune complex depleted sera. For removal of immune complexes, some sera were centrifuged at 100 000 g for one hour.

NHK were seeded in 96 well microtitre plates (20 000 cells/well) and left in an



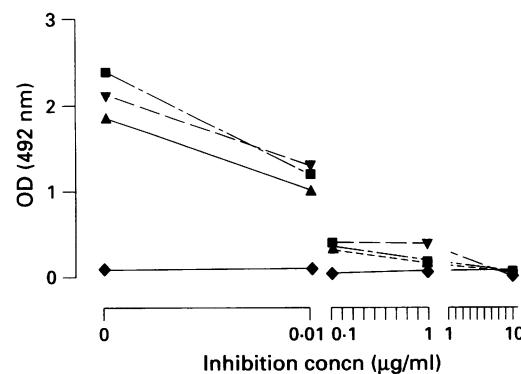
**Figure 1** Immunoblots, showing reactivity with 52 kDa, but not with 60 kDa, Ro(SS-A). LANE 1: Affinity purified anti-52 kDa Ro(SS-A); LANES 2 and 3: two monospecific anti-52 kDa Ro(SS-A) positive Sjögren's sera (pSS1, pSS2, respectively); LANE 4: serum pool derived from normal donors (n = 150 sera) (control).

atmosphere of 5% carbon dioxide. After a washing step, the cells were incubated with RPMI (Biochrom, Berlin, Germany) and 2% bovine albumin 100  $\mu$ l/well. Affinity purified antibodies to Ro(SS-A) 52 kDa and two sera from anti-52 kDa Ro(SS-A) positive patients (pSS1 and pSS2) (1:100 dilution in RPMI medium) were incubated with NHK which had been treated with recombinant TNF $\alpha$  4 ng/ml (Boehringer, Germany) for one, two, four, six, and 24 hours. Samples of sera from a pool of 150 blood donors (NHS) (diluted 1:100 in RPMI), monoclonal anti-La(SS-B) and anti-U1RNP 68 kDa, and mouse peroxidase conjugated antihuman IgG (diluted 1:500) and RPMI medium served as controls. After a washing step, 100  $\mu$ l/well of peroxidase coupled antihuman IgG (1:500 dilution) or antimouse IgG (1:200 dilution) were incubated for one hour, followed by another washing step. Antibody binding was detected enzymatically using *o*-phenylenediamine as substrate and a microplate reader at 492 nm. Optical density (OD) of NHK alone served as control.

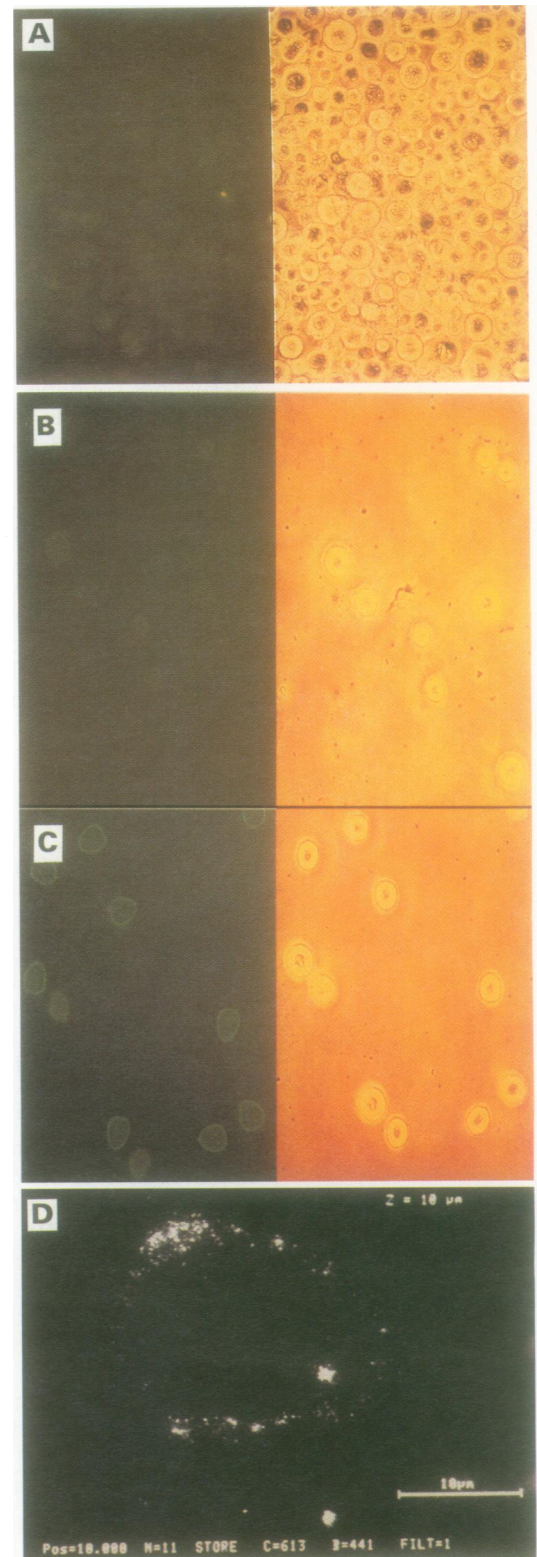
## Results

### SPECIFICITY OF SERA

Affinity purified 52 kDa Ro(SS-A) antibodies recognised the 52 kDa Ro(SS-A) antigen (derived from HEp-2 cells<sup>21</sup>) both in immunoblotting (fig 1) and in the ELISA. The selected sera pSS1 and pSS2 derived from primary Sjögren's patients were monospecifically positive for anti-52 kDa Ro(SS-A) (fig 1) as determined by immunoblotting, various enzyme immunoassays, and other routine methods. Of note, reactivity of a recombinant protein to the 60 kDa Ro(SS-A) protein of both sera and the affinity purified antibodies was not detectable by ELISA or immunoblotting. Preincubation of sera with recombinant 52 kDa Ro(SS-A) antigen reduced the antibody reactivity by up to 98% (affinity purified 52 kDa antibodies), 96% (pSS1), and 94.5% (pSS2) in an ELISA using a different 52 kDa recombinant<sup>17</sup> (fig 2). Control pooled sera from healthy blood donors was unreactive with any autoantigen tested.



**Figure 2** Inhibition ELISA of affinity purified antibodies to 52 kDa Ro(SS-A) ( $\blacktriangledown$ ) and two anti-52 kDa Ro(SS-A) monospecific Sjögren's sera ( $\blacksquare$ ,  $\blacktriangle$ ) preincubated with a recombinant 52 kDa antigen<sup>17</sup> before testing in the 52 kDa Ro(SS-A) ELISA system.  $\blacklozenge$  = Normal human serum pool (control).



**Figure 3** A: Cultured normal human keratinocytes (NHK), not preincubated with TNF $\alpha$ , dispersed in a cobble stone pattern, after indirect staining by antibodies to 52 kDa Ro(SS-A). B: Amino-3-propyltriethoxysilane (APES) fixed NHK not preincubated with TNF $\alpha$ , after indirect staining by antibodies to 52 kDa Ro(SS-A). Note weak staining. C: APES fixed NHK preincubated with TNF $\alpha$  for 90 minutes, showing enhanced membrane binding of affinity purified antibodies to 52 kDa Ro(SS-A) although, overall, 15–28% of TNF $\alpha$  pretreated cells did not show enhanced antibody binding. D: Fixed NHK cells preincubated with TNF $\alpha$  for 90 minutes, showing enhanced membrane staining by affinity purified antibodies to 52 kDa Ro(SS-A) antibodies. A–C: Light microscopy and indirect immunofluorescence (original magnification  $\times$ 400); D: Confocal laser scanning microscopy (original magnification  $\times$ 630).

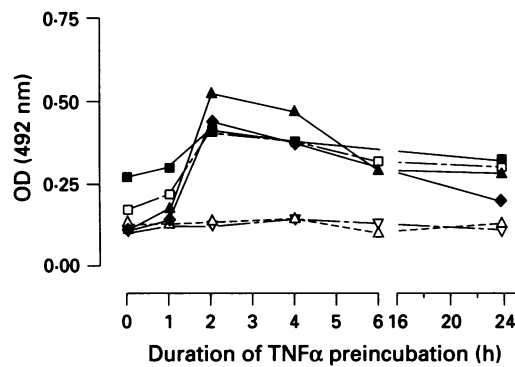


Figure 4 Cyto ELISA with unfixed cells. Surface binding of anti-52 kDa Ro(SS-A) (absorbance at 492 nm) using purified antibodies (I = ■, II = ▲, IV = ◆) and a monospecific patient serum (pSS2) (□) compared with controls (normal blood donor pool = Δ, peroxidase conjugated IgG = ▽). Each value represents the mean of three determinations (see table).

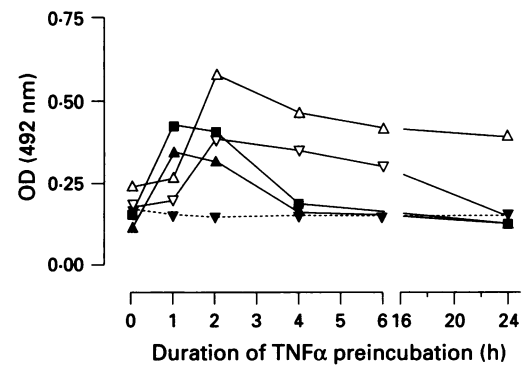


Figure 5 Cyto ELISA. Kinetics of membrane binding on the surface of TNF $\alpha$  pretreated NHK, by monoclonal antibody to La(SS-B) (experiments I = ■ and II = ▲; performed on different days), monoclonal antibody to U1RNP 68 kDa (▼), the monospecific anti-52 kDa Ro(SS-A) serum (pSS1) (Δ), and affinity purified antibodies to 52 kDa Ro(SS-A) (▽). Each value represents the mean of three determinations (see table).

ANTIGEN EXPRESSION

Indirect immunofluorescence with fixed NHK showed that expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens increased under the influence of TNF $\alpha$  (fig 3A-C). Interestingly, not all cells stained to the same intensity after TNF $\alpha$  stimulation. About 15-28/100 (mean 22.4%) cells did not show significant membrane staining by anti-52 kDa Ro(SS-A) or anti-La(SS-B) (fig 3C). Anti-52 kDa Ro(SS-A) binding on the surface of TNF $\alpha$  pretreated NHK was also confirmed using laser scanning microscopy (fig 3D). Anti-La(SS-B) simultaneously showed enhanced membrane binding after TNF $\alpha$  treatment. In contrast, neither the monoclonal antibodies to U1RNP 68 kDa nor the NHS serum showed cell surface staining on NHK.

Cyto ELISA with unfixed NHK demonstrated membrane expression of 52 kDa Ro(SS-A) and La(SS-B), but monoclonal antibody against U1RNP 68 kDa did not show reactivity (figs 4, 5). The kinetics of the expression of La(SS-B) antigen on the surface of NHK differed from those of the 52 kDa Ro(SS-A) antigen after preincubation with TNF $\alpha$ . Incubation with NHS, peroxidase conjugated IgG, AB serum, and RPMI did not result in increased antibody binding (controls). Background controls had OD values in the range 0.023 to 0.097 (SD 0.062).

After coincubation of NHK with TNF $\alpha$  4 ng/ml and with purified anti-52 kDa

Ro(SS-A) as probe, the 52 kDa antigen had a maximum cell surface expression after two hours and a slow decrease over 24 hours. In contrast, with a mouse monoclonal antibody, the La(SS-B) antigen was expressed rapidly by TNF $\alpha$  (within one hour) and showed a fast decrease to the preincubation value (over three hours) (fig 5). The monospecific sera pSS1 and pSS2 had kinetic profiles similar to those obtained with the affinity purified antibodies (figs 4, 5). The table summarises the results of three cyto ELISA measurements with unfixed cells, demonstrating the reproducibility of these kinetic profiles.

Discussion

In common with other investigators,<sup>22-25</sup> we have observed that unstimulated cells such as NHK express the 52 kDa Ro(SS-A) and La(SS-B) antigens solely within the nucleus; some studies have also demonstrated cytoplasmic binding of these antigens in fixed HEp-2 cells.<sup>26</sup> Autoantibodies to Ro(SS-A) and La(SS-B) most often occur simultaneously, which can be explained by the well recognised interaction of both target molecules.<sup>27 28</sup> Membrane expression of Ro(SS-A) and La(SS-B) has been shown to occur only under certain conditions.<sup>6-13 29</sup> Similarly, cell surface expression of other autoantigens also normally located in the nucleus

Median values (SD) of three cyto ELISA measurements with unfixed cells demonstrating the reproducibility of the time courses of expression of 52 kDa Ro(SS-A) and La(SS-B) antigen compared with controls

| Incubation time (h) | I             | II            | III           | IV            | pSS1          | pSS2          | mLaI          | mLaII         | NHS           | P-IgG         | Anti-U1RNP 68 kDa |
|---------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------|
| 0                   | 0.271 (0.055) | 0.108 (0.082) | 0.18 (0.048)  | 0.113 (0.068) | 0.242 (0.033) | 0.17 (0.03)   | 0.153 (0.065) | 0.111 (0.03)  | 0.13 (0.011)  | 0.098 (0.052) | 0.16 (0.03)       |
| 1                   | 0.299 (0.108) | 0.172 (0.038) | 0.194 (0.021) | 0.136 (0.013) | 0.265 (0.044) | 0.216 (0.012) | 0.429 (0.029) | 0.346 (0.063) | 0.124 (0.025) | 0.115 (0.024) | 0.15 (0.06)       |
| 2                   | 0.416 (0.076) | 0.528 (0.042) | 0.39 (0.122)  | 0.443 (0.04)  | 0.577 (0.125) | 0.409 (0.106) | 0.406 (0.071) | 0.32 (0.044)  | 0.132 (0.057) | 0.12 (0.035)  | 0.14 (0.02)       |
| 4                   | 0.381 (0.038) | 0.471 (0.188) | 0.353 (0.111) | 0.377 (0.047) | 0.469 (0.182) | 0.38 (0.163)  | 0.188 (0.037) | 0.169 (0.045) | 0.143 (0.048) | 0.139 (0.024) | 0.15 (0.0)        |
| 6                   | 0.294 (0.049) | 0.306 (0.051) | 0.306 (0.051) | 0.423 (0.07)  | 0.321 (0.088) | 0.321 (0.088) | 0.321 (0.088) | 0.321 (0.088) | 0.105 (0.028) | 0.123 (0.006) | 0.1 (0.04)        |
| 24                  | 0.324 (0.054) | 0.29 (0.075)  | 0.155 (0.096) | 0.194 (0.062) | 0.402 (0.096) | 0.305 (0.044) | 0.132 (0.064) | 0.135 (0.057) | 0.126 (0.035) | 0.108 (0.068) | 0.1 (0.18)        |

I-IV = Affinity purified 52 kDa Ro(SS-A) antibody fractions; pSS1 and 2 = monospecific anti-52 kDa sera from patients with Sjögren's syndrome; mLaI and II = repetitive test using monoclonal La antibodies; NHS = pooled serum from 150 healthy donors; P-IgG = peroxidase labelled anti-IgG; anti-U1RNP 68 kDa = monoclonal antibody control.



(DNA,<sup>30</sup> histone,<sup>31</sup> Ku,<sup>32</sup> and U1RNP<sup>6 9 33</sup>) can occur, suggesting an analogous mechanism. As NHK are capable of releasing significant amounts of biologically active TNF $\alpha$  in response to a wide variety of stimuli, including ultraviolet radiation, we examined the possibility that this cytokine may also play a part in membrane expression of the Ro(SS-A) and La(SS-B) antigens, implying a role of TNF $\alpha$  in the pathogenesis of photosensitive autoimmune diseases such as systemic lupus erythematosus. To our knowledge, our study is the first report demonstrating TNF $\alpha$  as an inducer of NHK surface expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens.

The lack of cell surface expression in the presence of TNF $\alpha$  in about 22% of the cells is an interesting finding and remains unexplained. Differences in TNF $\alpha$  receptor expression may render cells with fewer receptor molecules on their surface insensitive to a subsequent stimulation with the ligand, TNF $\alpha$ , and differences in cell cycle status and differential expression of other cytokines could also explain the lack of response in some cells.

Recently, it has been demonstrated that nuclear and cytoplasmic antigens are expressed at the cell surface during ultraviolet B light induced apoptosis.<sup>10</sup> The presence of Ro(SS-A) in small apoptotic blebs containing parts of the endoplasmic reticulum and in larger blebs could be detected at the NHK cell surface after exposure to ultraviolet B. The larger blebs also contained DNA, Ro, La, and small nuclear RNP (snRNP). Apoptotic events might contribute to the mechanism of surface expression of autoantigens, as TNF $\alpha$  is known to be a strong inducer of apoptosis.<sup>34 35</sup> Induction of apoptosis may be an important consequence of exposure to TNF $\alpha$ . This is supported by our further finding that cell viability was 95% at the start of the incubation (as determined by AO/EB staining), followed by a subsequent decrease in cell viability and a parallel increase in propidium iodine reactivity, indicating induction of apoptosis in the presence of TNF $\alpha$  (data not showing). It has also been suggested<sup>10</sup> that free oxygen radicals may play an important part in ultraviolet B induced autoantigen processing. As TNF $\alpha$  is also known to induce free oxygen radicals,<sup>35 36</sup> it is tempting to speculate that the enhanced cell membrane expression of Ro(SS-A) and La(SS-B) could be mediated, at least in part, via this mechanism.

In contrast to the findings of Casciola-Rosen *et al.*,<sup>10</sup> we were not able to detect cell surface expression of U1RNP 68 kDa antigen in our system after TNF $\alpha$  treatment. Casciola-Rosen's group have recently shown that this U1RNP protein is specifically cleaved in apoptotic cells, resulting in a 40 kDa fragment,<sup>33</sup> and it is possible that the monoclonal antibody we used did not recognise this particular antigen.

Well documented inducers of Ro and La surface expression include ultraviolet light,<sup>6-10</sup> oestradiol,<sup>8 11</sup> and adenovirus infection,<sup>12 13</sup> while injury, immunological (interferon gamma (IFN $\gamma$ ), IL-1 $\alpha$ , IL-2), inflammatory (leuko-

triene B4), metabolic (cholera toxin, phorbol myristate acetate) and hormonal (17- $\beta$  oestradiol, progesterone, dihydrotestosterone) stimuli failed to induce expression of Ro(SS-A).<sup>8</sup> Most interestingly, ultraviolet B induction of autoantigen expression was found to be significantly enhanced by oestradiol:<sup>8</sup> specifically, a twofold increase in Ro(SS-A) expression was detected compared with induction by ultraviolet B alone, and correlated with the preincubation time, but was not dose dependent. Because IL-1 is an important mediator of inflammatory skin diseases and is also released by NHK upon ultraviolet radiation, we examined the *in vitro* effects of IL-1 on the membrane expression of the 52 kDa Ro(SS-A) and La(SS-B) antigens (data not shown). Despite using several reaction conditions, we were unable to demonstrate induction of the expression of these autoantigens by IL-1. An infectious cause of the cellular distribution of La(SS-B) has previously been investigated in four different cell lines.<sup>13</sup> Only adenovirus infection induced an increase of La(SS-B) membrane expression after 48 hours. IFN $\gamma$  was capable of enhancing only nucleolar and cytoplasmic, but not cell surface, expression of the La(SS-B) antigen by HEP-2 cells.<sup>8 13</sup>

There is a direct correlation between the presence of antibodies to Ro(SS-A) and photosensitivity in SLE.<sup>5</sup> It is very likely that the increased antigen expression observed with ultraviolet B and, from our results, TNF $\alpha$  is an important factor in the exacerbation of SLE. A role for TNF $\alpha$  as a crucial mediator of Ro(SS-A) and La(SS-B) antigen expression is emphasised by the finding that the effect of ultraviolet B can be blocked by TNF $\alpha$  antibodies.<sup>37</sup> Our results suggest that binding of autoantibodies might play a direct pathogenic part in photosensitive inflammatory skin diseases. This is in accordance with previous findings in an animal model,<sup>38</sup> but is in contrast with other mechanisms of tissue damage that result from immune complex deposition on vascular structures, as in nephritis or the sicca syndrome.<sup>39 40</sup>

In conclusion, our finding that 52 kDa Ro(SS-A) and La(SS-B) antibodies bind to their antigens on the surface of TNF $\alpha$  pretreated NHK strongly suggests a possible pathogenic effect of these antibodies in dermatitis. This process could represent an important factor in antibody induction and in the clinical activation of photosensitive autoimmune dermatitis, through release of TNF $\alpha$  after exposure to the sun.

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