Antinuclear Autoantibodies and Lupus Nephritis in Transgenic Mice Expressing Interferon γ in the Epidermis

By John P. Seery,* Joseph M. Carroll,* Victoria Cattell,‡ and Fiona M. Watt*

*From the *Keratinocyte Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom; and the ‡Department of Histopathology, St. Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine, London WC2A 3PX, United Kingdom*

Summary

Systemic lupus erythematosus (SLE) is a potentially fatal non–organ-specific autoimmune disease that predominantly affects women. Features of the disease include inflammatory skin lesions and widespread organ damage caused by deposition of anti-dsDNA autoantibodies. The mechanism and site of production of these autoantibodies is unknown, but there is evidence that interferon (IFN) γ plays a key role. We have used the involucrin promoter to overexpress IFN-γ in the suprabasal layers of transgenic mouse epidermis. There was no evidence of organ-specific autoimmunity, but transgenic animals produced autoantibodies against dsDNA and histones. Autoantibody levels in female mice were significantly higher than in male transgenic mice. Furthermore, there was IgG deposition in the glomeruli of all female mice and histological evidence of severe proliferative glomerulonephritis in a proportion of these animals. Our findings are consistent with a central role for the skin immune system, acting under the influence of IFN-γ, in the pathogenesis of SLE.

Systemic lupus erythematosus (SLE) is a relatively common non–organ-specific autoimmune disease, with a prevalence comparable to that of multiple sclerosis (1). SLE predominantly affects women, the female/male ratio being ~9:1 (2). In this condition, splenomegaly and inflammatory skin lesions of varying severity occur in association with autoantibody production against a variety of nuclear antigens and multiple organ damage (1). Renal involvement complicates 60–70% of cases and its severity largely determines prognosis (3). The renal glomeruli are the prime site of injury due to the presence of anti-dsDNA autoantibodies in the mesangium and capillary walls (4). Anti-dsDNA antibodies intravenously administered to mice have been shown to induce glomerulonephritis (GN)[1], although the reason for tissue injury remains controversial (1, 4).

The mechanism of antinuclear autoantibody production is unknown, but there is evidence that IFN-γ plays a role. First, the emergence of SLE, with de novo anti-dsDNA antibody production, has been described in patients receiving systemic treatment with IFN-α or -γ (5, 6). Second, manipulation of the circulating amount and function of IFN-γ can profoundly alter the course of lupus in mouse models of the disease; systemic administration of IFN-γ accelerates the rate of progression to GN in lupus prone (NZB × NZW)F1 mice (7) and administration of anti–IFN-γ or soluble IFN-γ receptor to these animals can delay development of the disease (7, 8). Nevertheless, the precise effects of IFN-γ on murine lupus appear to be critically dependent on the dosage of the cytokine, the timing of administration, and the genetic background of the animal. For example, systemic administration of IFN-γ to lupus prone MRL/lpr-lpr mice has no effect on the course of the disease (9).

The site of production of pathogenic antinuclear autoantibodies in SLE is obscure. However, the observation that UV radiation, a factor known to exacerbate the disease, can induce translocation of nuclear antigens to the keratinocyte surface suggests that the skin immune system may be involved (10, 11). We have recently made transgenic mice in which IFN-γ is expressed in the suprabasal layers of the epidermis under the control of the involucrin promoter (12). This results in marked overexpression of IFN-γ in the epidermis but no increase in the level of IFN-γ in the blood. IFN-γ transgenic mice develop a nonblistering inflammatory skin disease with dermal edema and have marked splenomegaly. In addition, serum from the transgenic mice contains antibodies that produce a nuclear pattern of staining on sections of normal mouse epidermis (12). In view of these findings, we investigated IFN-γ transgenic mice for the presence of murine lupus.

[1]*Abbreviations used in this paper:* ENA, extractable nuclear antigens; GN, glomerulonephritis; HRP, horseradish peroxidase.

Materials and Methods

Preparation of Transgenic Mice. This was carried out as previously described (12). In brief, a transgene with the cDNA for murine IFN-γ under the control of the involucrin promoter was injected into fertilized oocytes from (CBA × C57/BL10)F1 mice. Three independent founder lines were generated: line 1205D contains 2 copies of the transgene, 1205C contains 6 copies, and 1212F contains 32 copies. The characteristics of the mice which were previously reported (12) and are reported here were observed in all three founder lines.

IFN-γ ELISA. Serum from 15 transgenic mice (8 males, 7 females) aged 4–13 mo was tested for the presence of IFN-γ using a murine IFN-γ Cytoscreen immunoassay kit (Biosource Intl., Camarillo, CA; reference 12). Recombinant mouse IFN-γ (Genzyme, Cambridge, MA) in the range of 10–500 pg/ml was used to standardize for known amounts of protein. In addition, extracts of renal tissue from two 7-mo-old transgenic female mice and two age- and sex-matched negative littermate controls were prepared by sonication on ice in 1 ml PBS containing 0.2 mM PMSF and 1 μM pepstatin. IFN-γ levels in both kidneys from each animal were measured using the IFN-γ Cytoscreen immunoassay kit and expressed per microgram of total protein present. Experiments were performed in triplicate and chromogenic results were read on a spectrophotometer at OD_{450}.

Indirect Immunofluorescence on Normal Mouse Skin and Esophagus. Serum samples from transgenic mice and negative control littermates were screened for the presence of autoantibodies by indirect immunofluorescence on normal mouse tail skin or esophagus. Unfixed, 8-μm-thick frozen sections of normal mouse skin/esophagus were allowed to air-dry at room temperature for 30 min before staining. Sections were blocked for 30 min in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ (PBSABC), and 10% FCS (Imperial Laboratories, Andover, UK) followed by incubation with mouse serum at a dilution of 1:10 in PBSABC for 45 min. Sections were then incubated for 45 min with Texas red–conjugated goat anti–mouse IgG (Jackson ImmunoResearch Labs, Inc., West Grove, PA) at a dilution of 1:100 in PBSABC.

Cell Culture. Isolation of human keratinocytes from newborn foreskin and cultivation on a feeder layer of mitomycin C–treated 3T3 cells have been previously described (13, 14). The culture medium consisted of one part Ham's F12 medium and three parts DMEM, supplemented with 1.8×10^{-4} M adenine, 10% FCS, 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 10^{-10} M cholera toxin, and 10 ng/ml epidermal growth factor. An established mouse keratinocyte line, provided by Dr. Rosario Romero (Imperial Cancer Research Fund) was grown under the same conditions as for human keratinocytes, except that the incubation temperature was 32 rather than 37°C.

Immunofluorescence Staining of Cultured Keratinocytes. Mouse and human keratinocytes grown on coverslips were fixed and permeabilized for 10 min using a 50:50 methanol/acetone solution at −20°C or in 3.7% formaldehyde for 10 min at room temperature, followed by 0.1% Triton X-100 in PBS for 5 min at room temperature. The same staining patterns were observed with each fixation technique. The coverslips were incubated with transgenic mouse serum diluted 1:10 in PBSABC for 45 min. After washing in PBS, coverslips were incubated with Texas red–conjugated goat anti–mouse IgG for 45 min. In some experiments, coverslips were incubated with rabbit antidesmoglein antibody 919 (recognizing the cytoplasmic repeat region of desmoglein-1 and -2, provided by Dr. Anthony Magee, National Institute for Medical Research, London, UK; reference 15), followed by Texas red–conjugated donkey anti–rabbit IgG (Jackson ImmunoResearch Labs.). All incubations and PBS washes were carried out at room temperature. Stained cells were mounted in Gelvatol and examined using an Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY).

Western Blotting. Confluent cultured mouse and human keratinocytes were lysed on ice in extraction buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.1% Triton X-100, 2 mM PMSF, and 0.01% leupeptin. The lysates were centrifuged at 14,000 rpm for 5 min and the pellets were discarded. The supernatants were subjected to SDS-PAGE on 6 or 8% slab gels, followed by transblotting to nitrocellulose paper. Transblotting was carried out in 48 mM Tris, 387 mM glycine, and 3.5 mM SDS containing 20% methanol for 2 h at 35 V followed by 2 h at 70 V. The blots were blocked overnight in 5% skim milk in PBS containing 0.01% Tween (PBS/T), then incubated for 1 h with serum from transgenic or control mice diluted at 1:200 in PBS/T containing 0.1% BSA (Sigma Chemical Co., Poole, Dorset, UK). Nitrocellulose strips were then incubated for 1 h with horseradish peroxidase (HRP)–conjugated rabbit anti–mouse Ig (DAKO, High Wycombe, UK) diluted 1:5,000 in PBS/T containing 0.1% BSA. Between antibody incubations, strips were washed three times with PBS/T. Additional strips were incubated with rabbit antidesmoglein antibody 145 (raised against the repeat region of desmoglein-1 and recognizing all three desmogleins, provided by Dr. Anthony Magee) followed by HRP-conjugated donkey anti–rabbit Ig (Amersham International, Little Chalfont, UK). All incubation and washing steps were performed at room temperature. The peroxidase activity was visualized by enhanced chemiluminescence (ECL; Amersham International).

Assay for Extractable Nuclear Antigens (ENA). Serum samples were screened for the presence of antibodies against extractable nuclear antigens (Sm, U1RNP, SS-A [Ro], SS-B [La], Jo1, and Scl-70) using a commercially available ENA screening counter current immunoelectrophoresis (CIE) kit (The Binding Site, Ltd., Birmingham, UK). Transgenic serum found to be positive on this screen was further characterized using an ENA typing CIE kit specific for the same antigens (The Binding Site, Ltd.). For ENA screening, 120 μl of ENA extract (buffered sheep spleen extract preserved in 100 mM PMSF and 10 mM mercaptoethanol) and 20 μl of test serum were applied to the surface of an agarose gel by means of an application mask. For ENA typing, 15–20 μl of test serum and 55–100 μl individual ENAs were impaired. For both procedures electrophoresis was carried out at 50 V for 75 min on a Beckman Paragon power pack. Gels were stained in Acid blue 29 in 5% vol/vol acetic acid for 2 s and destained in 5% vol/vol acetic acid for 5 min. Gels were dried completely by placing in a 45°C incudryer for 15 min and were then examined for a visible immunoprecipitate.

Crithidia luciliae Staining. Serum samples from transgenic and negative control littermates were screened for the presence of anti-dsDNA antibodies using a commercially available *C. luciliae* dsDNA kit (The Binding Site, Ltd.). In brief, slides coated with *C. luciliae* were incubated for 30 min at room temperature with serum samples diluted 1:10 in PBS. The slides were washed in PBS and then incubated for 30 min with FITC-conjugated goat anti–mouse IgG (Jackson ImmunoResearch Labs.). After further washing, the slides were mounted in Gelvatol (Monsanto Chemical Co., St. Louis, MO) and viewed using a confocal microscope.

Antihistone and Anti-dsDNA ELISA. The levels of antihis-

tone and anti-dsDNA antibodies in serum were measured using a modification of previously described methods (16). Calf thymus histones (Sigma Chemical Co.) were diluted in PBS to a concentration of 2.5 μg/ml and 0.2 ml of this antigen solution was added to each well of an Immulon II microtiter plate (Dynatech Labs., Inc., Chantilly, VA). After overnight incubation at 4°C, wells were coated with 0.4 ml gelatin (1 mg/ml in PBS) for at least 24 h at 4°C. After washing, 0.2 ml of serum samples diluted 1:1,000–1:4,000 in 0.1% Tween, 1 mg/ml gelatin, and 0.5% BSA in PBS were added and incubated for 1.5 h at room temperature. After washing, HRP-conjugated rabbit anti–mouse Ig (DAKO) diluted 1:4,000 in 0.1% Tween in PBS was added. After 1.5 h of incubation at room temperature, the wells were washed and substrate solution was added. The OD was then read with an automated spectrophotometer at 492 nm.

To measure anti-dsDNA antibody levels, wells were coated with dsDNA (Sigma Chemical Co.). To attach dsDNA, microtiter wells were first coated with poly-l-lysine (Sigma Chemical Co.) at 5.0 μg/ml in H_2O for 1.5 h at 37°C. After washing, dsDNA was added at 5.0 μg/ml in PBS and incubated overnight at 4°C. After washing, serum samples diluted 1:250–1:4,000 were added as described above.

All ELISA tests were run in triplicate. In addition, serum samples were checked for nonspecific binding to control wells lacking antigen. Plates were assessed for contamination with ssDNA using a serum sample known to be strongly positive for anti-ssDNA antibodies and negative for anti-dsDNA antibodies (provided by Tim Plant, Regional Immunology Department, Birmingham Heartlands Hospital, Birmingham, UK). Sera from four female MRL/lpr mice (~6-mo-old), known to be strongly positive for antinuclear antibodies, were used as positive controls in antihistone and anti-dsDNA ELISAs (provided by Dr. C.T. Ravirajan, Bloomsbury Rheumatology Unit, University College, London, UK). In both the antihistone and the anti-dsDNA ELISAs, autoantibody levels in transgenic mice were compared to those in littermate controls. ODs obtained with serum from different groups of animals were compared using the Mann-Whitney *U* test.

Immunofluorescence Detection of Kidney Deposits. Kidney tissue from transgenic mice and negative control littermates was snap-frozen in an isopentane bath cooled in liquid nitrogen. Frozen sections embedded in OCT (Tissue Tek™; Miles Inc., Elkhart, IN) were cut at 5–8 μm thickness. Sections were air dried and blocked for 30 min with goat serum. Sections were then incubated for 45 min with Texas red–conjugated goat anti–mouse IgG diluted at 1:100. Each antibody incubation was carried out at room temperature and was followed by thorough washing in PBS. Stained sections were mounted in Gelvatol and examined using a Zeiss Axiophot microscope.

Histology and Electron Microscopy. For light microscopy, kidney tissue was fixed in formalin, paraffin-embedded, and sections were stained with hematoxylin and eosin, or periodic acid Schiff. For electron microscopy, kidney tissue was fixed in 4% buffered glutaraldehyde at 4°C, post-fixed in osmium tetroxide, and ultra thin sections were stained with uranyl acetate and Reynold's lead citrate.

Results

Animals Studied. 34 transgenic animals and 12 littermate controls were examined. Tables 1 and 2 list the age and sex of each animal and summarize several of the pa-

Table 1. *Transgenic Mice Examined*

Animal	Sex	Age	Serum IFN-γ	IIF	Staining pattern on CK	Western blot	CL	ENA	Kidney DIF
			pg/ml						
1	M	9			N	−	+	−	+
2	M	5		+		−	+	−	
3	M	12	UN	+			+		
4	M	4	15						
5	M	4	UN						
6	M	5	40	+	N	−	+	−	−
7	M	12					+		++
8	M	9	UN	+		−	+		+++
9	M	9		+	N	−	+	−	−
10	F	12	UN				+		
11	F	7	UN	+	N	−	+		++
12	F	6			N	−	+	−	+
13	F	10					+		++
14	F	10	UN	+			+		+++
15	F	11	UN				+		
16	F	4	UN		N	−	+	Sm	+++
17		4		+	N	−	+	−	−
18	M	12	15				−		
19	M	5	30	+	N	−	+	−	+++
20	M	5		+	N	−	−	−	−
21	M	8		+					
22	M	8		+	N	−			+++
23	M	8		+					
24	M	9		+					
25	M	5		−					
26	M	4		−					
27	M	13					−		
28	F	3		−					
29	M	13	UN						
30	F	12	UN						
31	F	12	UN						
32	F	12							
33	F	7					+		
34	F	7					+		

Age is quoted in months. *M*, male; *F*, female; *IIF*, indirect immunofluorescence on normal mouse skin or esophagus; *CK*, cultured keratinocytes; *CL*, testing for anti-dsDNA on *C. luciliae*; *ENA*, antibodies against extractable nuclear antigens; *DIF*, direct immunofluorescence; *UN*, undetectable; *N*, nuclear staining on cultured mouse and/or human keratinocytes. On direct immunofluorescence of kidneys, glomerular staining intensity ranged from none (−) to mild/focal (+), moderate (++), or intense (+++). The presence of subendothelial-mesangial deposits was confirmed by electron microscopy in mice 13 and 16. There was histological evidence of severe proliferative GN in animals 12, 14, and 16 (see Table 3).

Table 2. *Negative Control Littermates of Transgenic Mice Examined*

Animal	Sex	Age	Serum IFN-γ	IIF	CL	Kidney DIF
			pg/ml			
C1	M	12		−	−	−
C2	F	10		−	−	−
C3	F	3		−	−	
C4	F	10		−	−	−
C5	M	13	UN	−	−	−
C6	M	13	UN	−	−	−
C7	M	13		+	−	
C8	F	12	UN		−	
C9	F	11	UN		−	
C10	M	4	UN		−	
C11	F	7			−	
C12	F	7			−	

Age is quoted in months. *M*, male; *F*, female; *IIF*, indirect immunofluorescence on normal mouse skin/esophagus; *DIF*, direct immunofluorescence; *CL*, testing for anti-dsDNA on *C. luciliae*; *UN*, undetectable. Absence of glomerular Ig deposits was confirmed by electron microscopy in mice C4 and C5.

rameters measured. The majority of animals previously analyzed were under 5 mo of age (12). However, the mean age of the transgenic mice and negative control littermates in this study was greater; 8.1 and 9.6 mo, respectively. None of the transgenic mice in the early study had detectable levels of circulating IFN-γ (12). We used the same ELISA method to test the serum of 15 of the present group of transgenic mice and 5 negative littermate controls (see Tables 1 and 2). All 5 controls and 11 of the transgenic animals had no detectable serum IFN-γ, but 4 male transgenic mice had small amounts (15–40 pg/ml; limit of detection in the assay is 5 pg/ml). We had previously reported that IFN-γ was readily detectable in skin of transgenic but not control mice, the 1212F line having the highest concentration (33 pg/cm^2; reference 12). We measured IFN-γ levels in kidney tissue extracts of two transgenic females from this founder line (Nos. 33 and 34, Table 1) and two littermate controls (C11 and C12, Table 2). The level of IFN-γ detected was 4 pg/μg total protein in the transgenic mice and 6 pg/μg in the controls.

IFN-γ Transgenic Mice Produce Non–organ-specific Antinuclear Autoantibodies. Previous studies in transgenic models have shown that local overproduction of IFN-γ can result in tissue-specific autoimmunity (17, 18). To test whether the autoantibodies in our transgenic mice were keratinocyte-specific we stained sections of mouse esophagus and skin in which keratinocytes, stromal fibroblasts, muscle cells, and endothelial cells could all be identified. Positive staining of all cell types was observed (Table 1 and data not shown). Serum from 14 out of 17 transgenic mice was positive. Serum from 6 out of 7 littermate controls was negative.

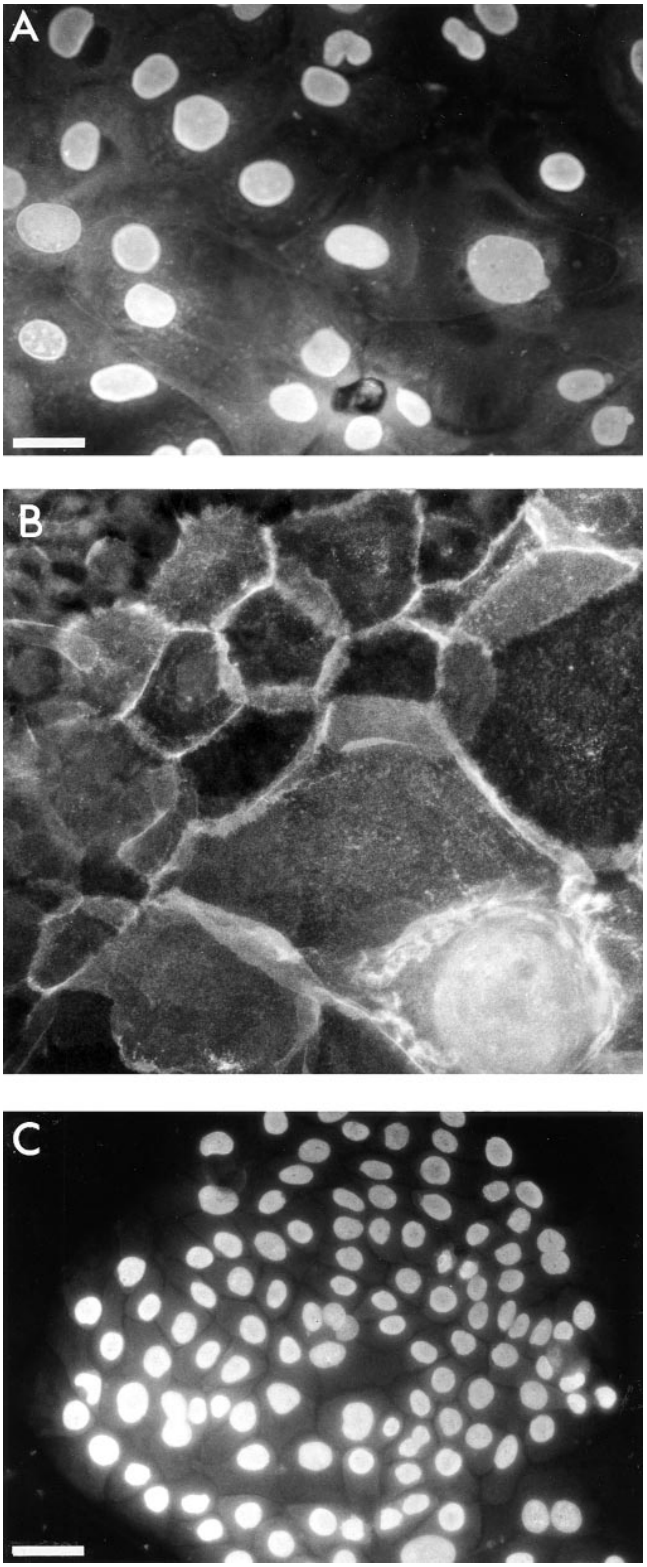

Figure 1. Indirect immunofluorescence staining of cultured (*A* and *B*) mouse and (*C*) human keratinocytes. (*A* and *C*) Stained with serum from a transgenic mouse (diluted 1:10). (*B*) Stained with antidesmoglein antibody. Scale bar (*A* and *B*): 30 μm; (*C*): 60 μm.

Autoantibodies directed against membrane antigens are a feature of several autoimmune skin diseases, the antigens frequently being proteins involved in cell–cell or cell–extracellular matrix adhesion (19, 20). The cellular distribution of the antigens recognized by antibodies in the serum of the IFN-γ transgenic mice was examined by staining cultured mouse and human keratinocytes (Fig. 1, *A* and *C*). In the 10 serum samples examined (Table 1) there was intense staining of the nucleus, with no evidence of membrane staining. Autoantibodies stained nuclei of both mouse and human cells. For comparison, keratinocytes were stained with an antibody to desmogleins, the autoantigens of pemphigus vulgaris and pemphigus foliaceus; as illustrated in Fig. 1 *B* the staining pattern was quite distinct from that observed with autoantibodies from the IFN-γ transgenic mice.

Antinuclear Antibodies in IFN-γ Transgenic Mice Are Specific for dsDNA and Histones. To identify proteins recognized by the autoantibodies from IFN-γ transgenic mice, extracts of cultured mouse and human keratinocytes were resolved on SDS-PAGE and subjected to immunoblotting (Table 1 and data not shown). 10 of the 12 serum samples recognized a single band with an apparent molecular mass of ~100 kD. However, serum from two nontransgenic BALB/c control mice also recognized the band, indicating that it was not specific to the transgenic mice. As predicted from the immunofluorescence staining shown in Fig. 1, the 100-kD band was not recognized by a pandesmoglein antibody (data not shown). Furthermore, the mobility is distinct from that of the bullous pemphigoid antigens (21).

Next, we tested the serum samples for antibodies to ENA using counter-current immunoelectrophoresis. Increased levels of autoantibodies against the ENA screened (Sm, U1RNP, SS-A [Ro], SS-B [La], Jo1, and Scl-70) were detected in one animal only (no. 16 in Table 1). On further characterization this mouse was found to be positive for anti-Sm autoantibodies.

To examine whether antibodies to dsDNA were present, we screened serum for reactivity to the kineoplast of the flagellate organism *C. luciliae*. Indirect immunofluorescence testing of serum on *C. luciliae* has been used as a specific test for the presence of anti-dsDNA autoantibodies (22). Serum samples from 21 transgenic mice and 12 negative littermate controls were tested. 18 samples from transgenic animals produced definite staining of the kinetoplast (see Fig. 2 *A* and Table 1). All littermate controls were negative on this test (Table 2).

We used ELISA assays to quantitate the levels of anti-dsDNA autoantibodies and to determine whether antihistone antibodies were also present. Sera from male (mice Nos. 1–9 and 19; mean age, 7.4 mo; Table 1) and female transgenic mice (mice Nos. 10–16; mean age, 8.6 mo; Table 1) and negative control littermates (mice C1–C6 for anti-dsDNA and mice C1–C4, C9, and C10 for antihistone, Table 2), were measured and are shown in Fig. 2, *B* and *C* and Table 3. Compared to littermate controls (mean OD, 0.038) both male (mean OD, 0.081; $P < 0.01$) and female (mean OD, 0.232; $P < 0.003$) transgenic mice showed evidence of anti-dsDNA antibody production. Levels were significantly higher in females than males ($P < 0.007$). All four MRL/lpr mice, included as positive controls, produced higher levels of anti-dsDNA antibody than the transgenic females (mean OD, 0.848 vs. 0.232, respectively, Fig. 2 *B*). Female transgenic mice tested produced antihistone antibodies at levels comparable to MRL/lpr controls (mean OD, 0.313 and 0.478, respectively, Fig. 2 *C*). Antihistone antibody levels in serum from male transgenic mice did not differ significantly from negative littermate controls (mean OD, 0.011 and 0.013, respectively, Fig.

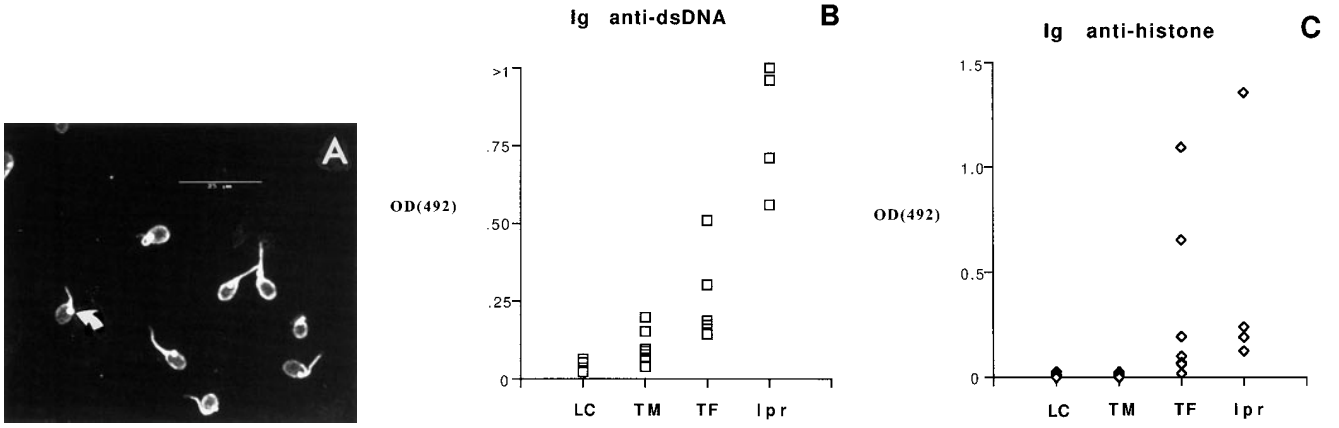

Figure 2. Screening serum for anti-dsDNA and antihistone autoantibodies. (*A*) *C. luciliae* assay. Staining with serum from a transgenic mouse results in fluorescence of the kinetoplast (*arrow*) of each organism. A series of 1-μm optical sections through the specimens was obtained with a confocal microscope and a composite image (Z series) was constructed. Scale bar: 10 μm. (*B*) Anti-dsDNA and (*C*) antihistone autoantibody levels in transgenic mouse serum. Sera from 17 transgenic mice and 6 negative littermate controls were tested individually against dsDNA at 1:250 and histones at 1:1,000 dilution. The OD value for each sample represents the mean of three measurements. *LC*, Littermate controls; *TM*, transgenic males; *TF*, transgenic females. Four MRL/lpr mice known to produce high levels of antinuclear antibodies are included as positive controls. A serum sample known to be positive for anti-ssDNA showed no significant binding in the dsDNA ELISA (OD 0.00 ± 0.05). All sera were tested on uncoated ELISA plate plastic. Nonspecific binding to plastic was low (OD on uncoated wells ranged from 0 to 5% of values on antigen-coated wells).

Table 3. *Kidney Pathology and Relative Levels of Antinuclear Antibodies in Female IFN-γ Transgenic Mice*

Animal	Anti-dsDNA	Antihistone	Kidney pathology
10	0.143	0.019	Mild mesangial GN
11	0.186	0.194	Mild mesangial GN
12	0.148	0.070	Severe diffuse proliferative GN
13	0.160	0.653	Mild mesangial GN
14	0.303	0.101	Severe diffuse proliferative GN
15	0.173	1.094	Mild mesangial GN
16	0.510	0.063	Severe diffuse proliferative GN

Antibody levels are expressed as OD measured at 492 nm. Anti-dsDNA and antihistone ELISAs were carried out at dilutions of 1:250 and 1:1,000, respectively.

2 *C*). Antinuclear autoantibodies were detected in female animals from all three transgenic founder lines. Interestingly, mice that produced the highest levels of anti-dsDNA did not necessarily produce high levels of antihistone antibodies and vise versa (Table 3).

IFN-γ Transgenic Mice Have Renal Immune Complex Deposition and GN. Anti-dsDNA antibodies are known to deposit in the kidneys of 60–70% of SLE patients and to cause GN (3, 4). We therefore examined the kidneys of the transgenic mice for evidence of autoantibody deposition and organ damage (Fig. 3). Immunohistochemistry of kidneys showed dense deposits of IgG within the glomeruli in all female IFN-γ transgenic mice examined ($n = 5$, Table 1). As in human lupus-nephritis, both mesangial (Fig. 3 *A*) and capillary (Fig. 3 *B*) patterns of Ig deposition were found. Five out of eight male mice tested had evidence of Ig deposits in the glomeruli (Table 1).

Histological examination of kidney tissue (16 males and 11 females) demonstrated clear evidence of GN in female mice only. The severity of the lesion varied from mild mesangial nephritis (results not shown) to severe diffuse proliferative GN (Fig. 3 *C*). The former was observed in mice from two founder lines, 1205C and 1212F, and overall occurred in ~25% of female animals examined (Nos. 12, 14, and 16, Tables 1 and 3). Subendothelial-mesangial deposits were confirmed by electron microscopy (Fig. 3 *E*) and the immunopathology closely corresponded with the findings in spontaneous murine lupus–like syndromes (23). Interestingly, severe proliferative GN occurred in female transgenic mice with high levels of anti-dsDNA (Table 3). Control littermates had normal kidneys ($n = 10$; Fig. 3 *D*).

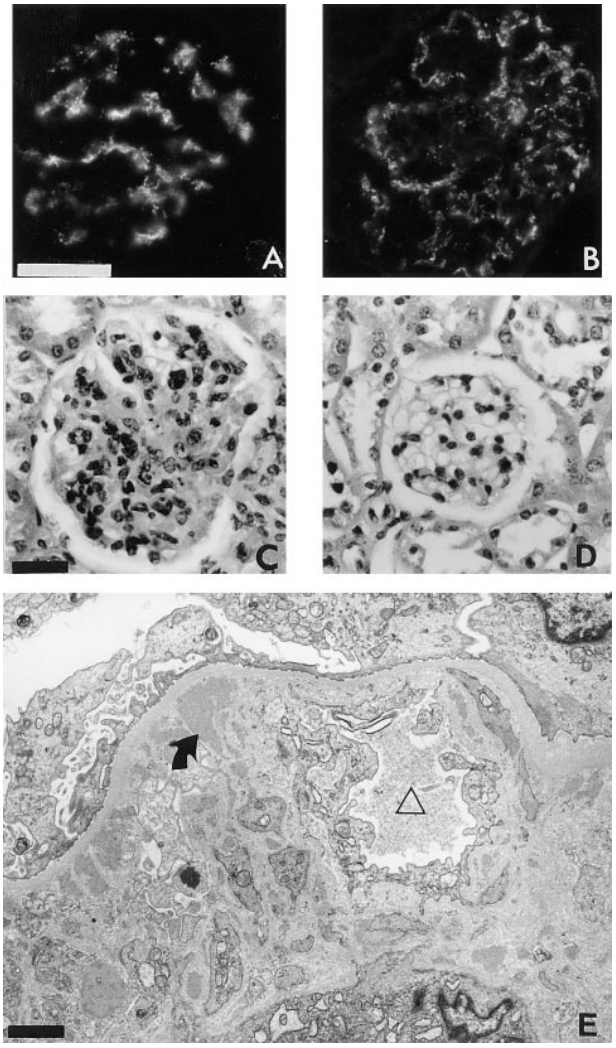

Figure 3. Glomerular immunopathology in female transgenic mice. Immunofluorescence staining of kidney tissue showing deposits of IgG in the mesangium (*A*) and globally on capillary walls (*B*). Hematoxylin and eosin stained glomerulus from a 10-mo-old female transgenic mouse (*C*) and a glomerulus from an age- and sex-matched negative littermate control (*D*). In *C*, note diffuse proliferative GN with hypercellularity, nuclear fragmentation, and reduction in capillary lumens. There were no significant tubulointerstitial or vascular lesions. (*E*) Electron micrograph of kidney tissue from a 4-mo-old female transgenic mouse shows electron-dense deposits in a subendothelial-mesangial distribution (*arrow*) and a narrowed capillary lumen (△). Scale bar (*A–D*): 60 μm; (*E*): 1.5 μm.

Discussion

We have shown that targeting an IFN-γ transgene to the suprabasal epidermal layers via the involucrin promoter results in production of antihistone and anti-dsDNA autoantibodies and in immune complex deposition in the kidneys. Autoantibody levels were higher in female mice than males and kidney damage was only found in females. These observations, together with our earlier findings that the mice had inflammatory skin lesions, dermal edema, and splenomegaly (12) suggest that our transgenic mice are a useful model for SLE. Reevaluating the skin phenotype of the transgenic mice in the light of this interpretation, the occasional separation of epidermis from dermis with infiltration of hemopoietic cells is probably the hydropic degeneration of basal cells that is characteristic of SLE (24).

The reason for the higher rate of SLE in females remains unknown. However, the defects in experimental tolerance which have been described in spontaneous murine lupus

are critically dependent on sex hormones, with androgens exerting a protective effect (for review see reference 25). In IFN-γ transgenic mice, the highest levels of both antihistone and anti-dsDNA autoantibodies were detected in female animals (Fig. 2, *B* and *C*). In addition, histological evidence of kidney pathology was only observed in female transgenic mice. There was no correlation between antihistone levels and GN severity. Furthermore, while the two female mice with highest levels of anti-dsDNA antibodies had marked immune complex deposition in the glomeruli (Table 1) and severe GN (Table 3), the correlation between anti-dsDNA levels and kidney damage was not absolute (compare mice 11 and 12, Table 3). This phenomenon is well described in patients with SLE. The extent of tissue immune complex deposition, and the degree of organ damage, do not depend solely on the serum titer of anti-dsDNA antibodies but also on several qualitative properties of the antibodies (26) and possibly on the host's ability to process immune complexes (27).

Previous observations in transgenic mice in which IFN-γ is overexpressed in specific tissues have suggested that local overproduction of IFN-γ is involved in the pathogenesis of organ specific autoimmunity (17, 18). In contrast, our transgenic mice did not produce autoantibodies characteristic of cutaneous autoimmune disease. Autoantibodies in our mice reacted with multiple cell types on tissue sections and did not recognize membrane proteins, including the autoantigens of pemphigus vulgaris or bullous pemphigoid, the two major autoimmune skin disorders; instead, they recognized nuclear antigens. In the light of the earlier transgenic studies, the lack of organ-specific autoimmunity in our transgenic system may appear surprising. However, previous studies have strongly implied that the consequences of IFN-γ overexpression may vary with tissue type. Transgenic expression of IFN-γ in beta-cells results in a cell-mediated immune destruction of pancreatic islets (17), whereas overexpression in the neuromuscular junction elicits a humoral response with no evidence of cell-mediated damage (18). It has been argued that the response of APCs to cytokines may differ in different tissue types, leading to different patterns of T cell activation (28). Certainly, there is evidence that the skin immune system has distinctive properties, both in terms of its APC and keratinocyte functions (29) and these may explain the difference in response to IFN-γ overexpression in the skin compared to other transgenic model systems.

With the exception of one animal, increased levels of anti-dsDNA antibodies were not accompanied by production of autoantibodies against the ENA tested. Interestingly, the single mouse with anti-ENA antibodies was found to be positive for anti-Sm autoantibodies, a serological finding considered highly specific for SLE (30). However, the consistent generation of high levels of antihistone and anti-dsDNA antibodies in our transgenic mice in the absence of significant autoreactivity to ENA supports the concept that different pathogenic mechanisms underlie the generation of the two types of autoantibody (31).

There is strong evidence that presentation of nuclear antigens to CD4-positive T cells is involved in the production of pathogenic anti-dsDNA autoantibodies (1) and Desai-Mehta et al. have isolated and characterized a subset of autoimmune T helper cells from patients with SLE (32) which strongly induce anti-dsDNA antibody production. Immunological abnormalities in the skin of IFN-γ transgenic mice suggest two possible mechanisms of autoantigen presentation to T cells. IFN-γ transgenic mice demonstrate a marked alteration in Langerhans cell distribution, consistent with migration from the epidermis to the dermis and the draining lymph nodes (12). These "professional" APCs could interact with autoreactive T cells in either site. However, transgenic mice also markedly upregulate keratinocyte MHC class II and intracellular adhesion molecule–1 expression and there is evidence of low level CD4-positive lymphocyte migration into the epidermis in some animals (12). Therefore, it is possible that keratinocytes play the key role in autoantigen presentation. It is known that IFN-γ can induce translocation of nuclear antigens from the nucleus to the cytoplasm in epithelial cells and that keratinocytes may act as APCs under certain conditions (33, 34). Keratinocytes in IFN-γ transgenic mice do not express the costimulatory molecule B7 (12). However, antigen presentation to T cells in the absence of costimulatory molecules could trigger an aberrant autoreactive immune response and, indeed, defective antigen presentation is a feature of SLE (35).

Skin lesions are one of the classical clinical manifestations of SLE. Our findings are consistent with a central role for IFN-γ and the skin immune system in the pathogenesis of the systemic complications of the disease. Elucidation of the precise mechanisms involved in the generation of antinuclear autoantibodies by the skin immune system in these animals may give valuable insights into the pathogenesis of SLE.

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Address correspondence to Dr. Fiona M. Watt, Keratinocyte Laboratory, Imperial Cancer Research Fund,

44 Lincoln's Inn Fields, London WC2A 3PX, UK. Phone: 44-171-269-3528; FAX: 44-171-269-3078. Joseph M. Carroll's current address is Genetics Institute, Andover, MA 01810.

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