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Intercellular Adhesion Molecule-1 (ICAM-1) Expression Is Upregulated in Autoimmune Murine Lupus Nephritis

Rudolf P. Wuthrich,* Anthony M. Jevnikar,* Fumio Takei,† Laurie H. Glimcher,‡ and Vicki E. Kelley*

From the Laboratory of Immunogenetics and Transplantation, Department of Medicine, Brigham and Women's Hospital, and the Harvard Center for the Study of Kidney Diseases, Harvard Medical School, Boston, Massachusetts; the Terry Fox Laboratory,† British Columbia Cancer Research Centre, Vancouver, British Columbia; the Department of Cancer Biology,‡ Harvard School of Public Health, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts*

Intercellular adhesion molecule-1 (ICAM-1) is a cell-surface protein regulating interactions among immune cells. To determine whether altered expression of ICAM-1 occurs in autoimmune lupus nephritis, we studied ICAM-1 expression in kidneys of normal and autoimmune MRL-lpr and (NZB× NZW)F_1 (NZB/W) mice. By immunoperoxidase staining, ICAM-1 is constitutively expressed at low levels in proximal tubules (PT), endothelium and interstitial cells in normal C3H/FeJ mice. In nephritic MRL-lpr and NZB/W kidneys, staining for ICAM-1 is increased in the PT, particularly in the brush border, and is prominent in the glomerular mesangium and the endothelium of large vessels. By Western blot analysis, ICAM-1 is not detected in the urine of normal BALB/c and C3H/FeJ or autoimmune MRL-lpr. By Northern blot analysis, nephritic MRL-lpr and NZB/W have a two- to fivefold increase in steady state levels of ICAM-1 transcripts in the kidney as compared with normal or prenephritic mice. This is paralleled by an increase in MHC class II transcripts. In cultured PT cells, ICAM-1 is expressed at basal levels in PT and is increased by the cytokines interferon-γ, IL-1α, and TNF-α. Thus cytokine-mediated upregulation of ICAM-1 in lupus nephritis may promote interaction of immune cells with renal tissue. The predominant apical expression of ICAM-1 opposite to the basolateral Ia expression suggests a novel role for this adhesion molecule in PT. (Am J Pathol 1990, 136:441–450)

Intercellular adhesion molecule 1 (ICAM-1) is an important cell-surface glycoprotein regulating interactions among immune cells and between accessory and T cells.[1–7] The interaction of ICAM-1 with target cells occurs through its ligand lymphocyte function-associated antigen 1 (LFA-1).[8–10] ICAM-1 has a wide tissue distribution[11] and is a member of the immunoglobulin superfamily.[12,13] To date, most research has been done in humans, in whom ICAM-1 is expressed on immune cells,[11] endothelial cells,[14,15] dermal fibroblasts,[11] keratinocytes,[16,17] and synovial cells.[18] The tissue distribution of ICAM-1 parallels the distribution of major histocompatibility complex class II (Ia) antigens.[11] In different cell types, ICAM-1 expression is upregulated by cytokines, including interferon-γ, IL-1, and TNF-α.[6,7,14–17,19] Recently, the cDNA encoding the murine ICAM-1 molecule has been isolated[20,21] and a monoclonal antibody (MAb) for the murine ICAM-1 has been identified,[22] thus allowing studies of ICAM-1 expression in murine models.

In the present study we investigated whether ICAM-1 was expressed in normal murine kidney, and if increased expression occurred in autoimmune MRL-*lpr* and (NZB×NZW)F_1 (NZB/W) mice. We have already reported increased MHC class II expression in MRL-*lpr* mice with lupus nephritis,[23] particularly in the proximal tubules (PT). We therefore determined whether ICAM-1 expression paralleled altered Ia expression and whether cytokines such

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Address reprint requests to Vicki E. Kelley, PhD, Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

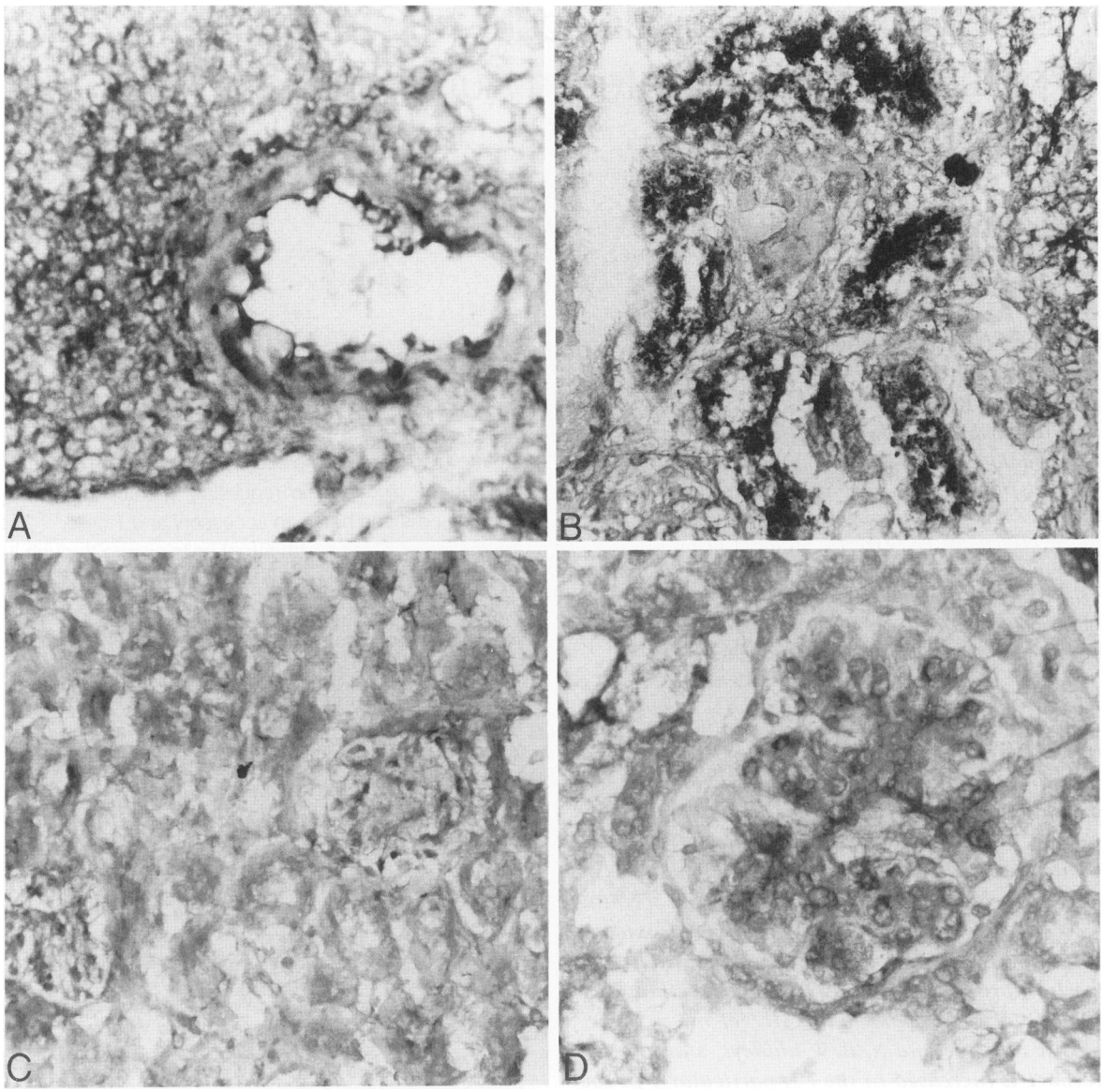

Figure 1. *ICAM-1 is detected by immunoperoxidase in MRL-lpr kidneys.* **A:** *Positive staining in mononuclear infiltrate and endothelium in MRL-lpr (× 1000).* **B:** *Proximal tubular ICAM-1 expression, localized mainly on brush border (MRL-lpr, × 660).* **C:** *Weakly positive PT and negative glomeruli from C3H/FeJ mouse (× 800).* **D:** *Positive mesangial staining in MRL-lpr glomerulus (× 1000).*

as TNF-α and IL-1, which are known to be overproduced in MRL-*lpr* and NZB/W lupus nephritis,[24,25] play a role in ICAM-1 expression. We detect constitutive ICAM-1 expression in PT and in the endothelium of normal mice, which is increased in nephritic MRL-*lpr* and NZB/W mice, mainly on the brush border of PT, and also in the glomerular mesangium. *In vitro*, ICAM-1 is expressed in cultured proximal tubular cells and can be upregulated by IFN-γ, TNF-α, and IL-1α. Thus cytokine-mediated ICAM-1 expression may contribute *in vivo* to the autoimmune process in the kidney.

Materials and Methods

Mice

Normal C3H/FeJ (H-2^k) and BALB/c mice (H-2^d), congenic MRL/MpJ−+/+ (MRL−++) (H-2^k) and autoimmune MRL/MpJ-*lpr/lpr* (MRL-*lpr*) (H-2^k), and (NZB× NZW)F1 (NZB/W) (H-2^{d,z}) mice (all female) were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained in our animal facility on standard laboratory chow. For immunohistologic studies, mice were killed at various

Table 1. *Immunoperoxidase Staining for ICAM-1 and Ia in Kidney**

Strain	Age (months)	Histology‡ (0–4)	ICAM-1/Ia expression†									
			PT§		Glomeruli		Interstitium		MNI§		Endothelium	
			ICAM-1	Ia	ICAM-1	Ia	ICAM-1	Ia	ICAM-1	Ia	ICAM-1	Ia
C3H/FeJ	4–6	0	1	<0.5	<0.5	0	1	1	—	—	1	0
MRL-++	4–15	0.5	1	<0.5	1	<0.5	1	1	0.5	0.5	1.5	<0.5
MRL-lpr	2–4	1.5	1.5	1	1	0	1	1	1.5	1.5	1.5	1
MRL-lpr	4–7	3	2.5	2.5	2.5	1	1.5	1.5	2.5	2.5	2	2
NZB/W	1.5	0.5	0.5	0.5	<0.5	<0.5	1	1	1.5	1	1.5	<0.5
NZB/W	8–9	2.5	2.5	1	2.5	1	1.5	1.5	2.5	2.5	2	1.5

* Cryostat sections (4 μm) were stained by immunoperoxidase for both ICAM-1 (MAb YN1/1.7.4) and Ia (MAb 10-3.6.2 for MRL-lpr, and MK-D6 plus 10-3.6.2 for NZB/W).

† ICAM-1 and Ia expression was scored on a scale from 0–3: 0, not detectable; 1, weak; 2, moderate; 3, strong staining. The mean score from 2–5 animals/group is reported.

‡ A histopathological score ranging form 0 to 4 was used to assess the degree of renal injury (0, normal; 1, small increase of cells within mesangium; 2, more pronounced mesangial proliferation and perivascular infiltration in cortex and medulla; 3, lobular formation of glomerulus, and prominent perivascular mononuclear infiltration; 4, glomerular crescent formation, presence of sclerotic glomeruli, tubular atrophy casts and vasculitis).

§ PT denotes proximal tubules; MNI, mononuclear infiltrates.

ages and kidney sections were snap frozen in OCT compound (Miles Inc., Elkhart, IN).

Materials

Monoclonal antibodies 10-2.16, 10-3.6.2 (both anti I-A^k) and MK-D6 (anti I-A^d) were purified from hybridoma supernatants using protein A-Sepharose CL-4B columns (Pharmacia, Piscataway, NJ). The rat anti-murine ICAM-1 mAb YN1/1.7.4 was purified by ammonium sulfate precipitation of the IgG fraction from ascites fluid, followed by DEAE Affigel Blue (Biorad, Richmond, CA) affinity chromatography.[22] All antibodies were biotinylated using a standard protocol. Recombinant murine interferon-γ and tumor necrosis factor-α were kindly provided by Genentech (South San Francisco, CA). Recombinant murine interleukin-1α was donated by Hoffmann-LaRoche (Nutley, NJ) and Pfizer (Groton, CT). Supernatants from J558L cells transfected with the murine IL-4 gene (gift of Dr. R. Tepper, Harvard Medical School) were used as a source for rIL-4.[26] Lipopolysaccharide (LPS, *S. minnesota*) was from Calbiochem (Behring Diagnostics, La Jolla, CA).

Cell Cultures and Cell Lines

Single-cell suspensions of renal cortical tubular cells from C3H/FeJ mice, predominantly of proximal tubular origin, were grown in primary culture after collagenase disper-

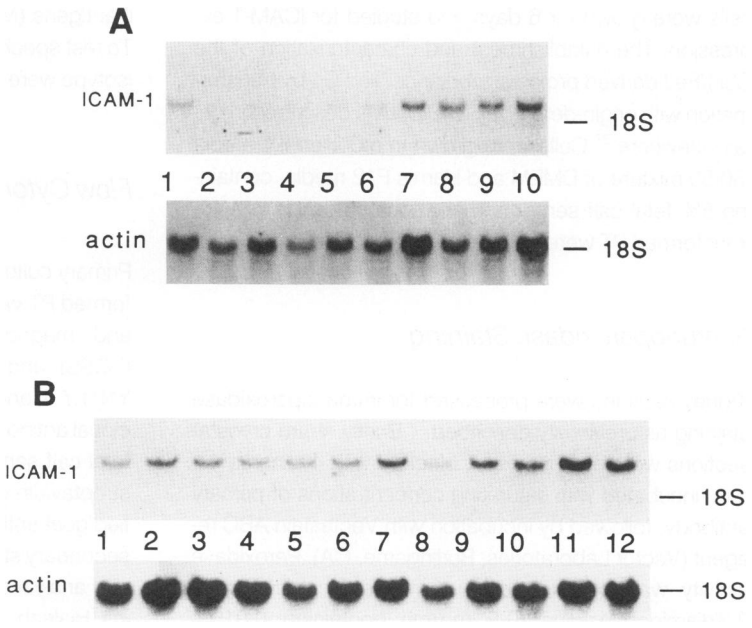

Figure 2. *Northern blot analysis of ICAM-1 gene expression in the kidney of C3H/FeJ, MRL-++ and MRL-lpr mice. Two blots were probed in separate experiments for ICAM-1 (top lanes) and were rehybridized with β actin on separate occasions (bottom lanes).* **A:** *Lanes 1–2, C3H/FeJ (4 and 6 months); lanes 3–4, MRL-++ (3 and 6 months); lanes 5–10, MRL-lpr (2, 6, 6, 7, 7 and 7 months).* **B:** *Lanes 1–3, C3H/FeJ (3, 5 and 6 months); lanes 4–5, MRL-++ (4 and 7 months); lanes 6–12, MRL-lpr (2, 2, 2, 3, 7, 7 and 9 months). See Table 2 for densitometric analysis of blots.*

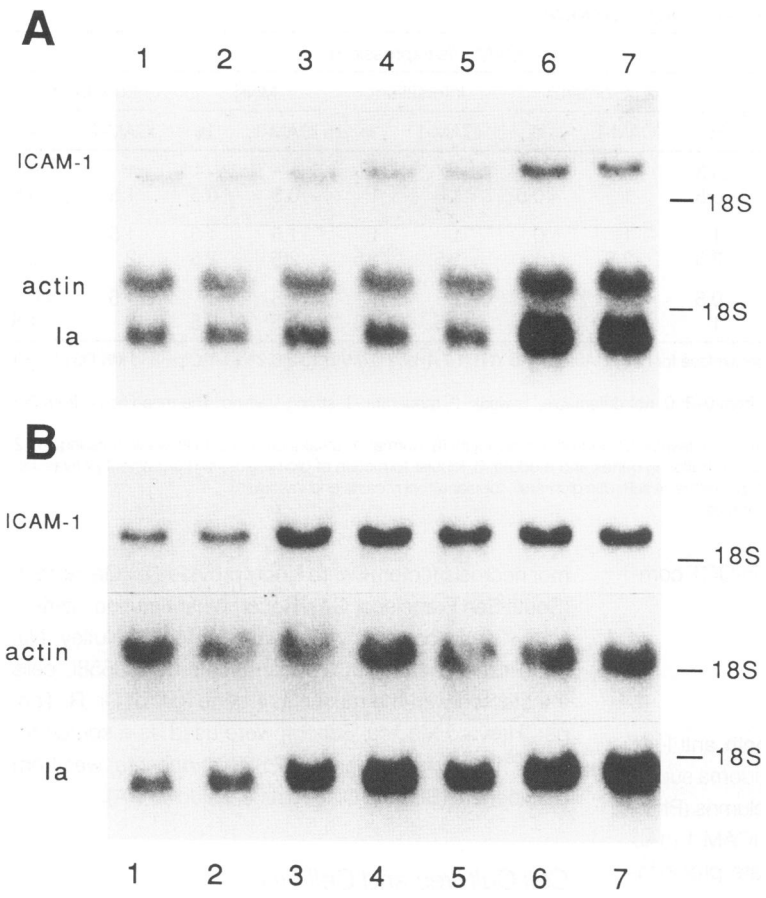

Figure 3. *Northern blot analysis of ICAM-1 and I-A_α gene expression in NZB/W kidneys. Two blots were probed in separate experiments for ICAM-1 (top lanes), Ia (A_α) (bottom lanes) and β actin (center lanes).* **A)** *Lanes 1–7, NZB/W (1.5, 1.5, 4, 4, 4, 8, and 8 months, respectively).* **B)** *Lanes 1–7, NZB/W (1.5, 4, 5.5, 8, 9, 9, and 9 months). See Table 3 for densitometric analysis of blots.*

sion of cortical tissue and sequential sieving, as previously described.[27] These cells display strong staining for alkaline phosphatase, a marker enzyme of PTs. The cells were grown for 6 days and studied for ICAM-1 expression. The establishment and characterization of the C3H/FeJ-derived proximal tubular cell line C1 by transformation with origin-defective SV40 DNA is described in detail elsewhere.[27] Cells were grown in modified K1 media (50:50 mixture of DMEM and Ham's F12 media, containing 5% fetal calf serum and hormonal additives). SV40-transformed PT were passaged by trypsinization.

Immunoperoxidase Staining

Kidney sections were processed for immunoperoxidase staining as previously described.[23] Briefly, 4-μm cryostat sections were acetone-fixed, blocked with 4% horse serum, incubated with saturating concentrations of primary antibody, followed by incubation with Vectastain ABC reagent (Vector Laboratories, Burlingame, CA). Peroxidase activity was detected by a 4-minute incubation with 3,3'diaminobenzidine (0.5 mg/ml) containing 0.015% H_2O_2. Sections were counterstained with methyl green and alcian blue. All sections were stained at the same time for both ICAM-1 (MAb YN1/1.7.4) and for MHC class II antigens (MAb 10-3.6.2 or MK-D6) on separate slides. To test specificity of the MAb, irrelevant MAb of the same isotype were used routinely, resulting in no staining.

Flow Cytometric Analysis

Primary cultures of proximal tubular cells and SV40-transformed PT were briefly trypsinized, washed with calcium- and magnesium-free Hanks balanced salt solution (HBSS), and stained for ICAM-1 using biotinylated MAb YN/1.7.4, and for MHC class II using MAb 10-2.16. Monoclonal antibodies were prepared at optimal dilutions in 3% fetal calf serum in HBSS. Phycoerythrin (PE)-conjugated streptavidin or fluorescein (FITC)-conjugated affinity-purified goat anti-mouse IgG $F(ab')_2$ fragment were used as a secondary step. Cells were fixed in 2% paraformaldehyde and analyzed on an Epics C cell sorter (Coulter Electronics, Hialeah, FL).

Table 2. *Densitometric Analysis of ICAM-1 Gene Expression in MRL-lpr Kidney**

Blot	Lane	Strain	Age (month)	U_p† (mg/d)	Histology§ (0–4)	Relative densitometric unit ratios‖ ICAM-1/actin ($\times 10^2$)
A	1	C3H/FeJ	4	—	—	1.4
	2	C3H/FeJ	6	—	—	1.0
	3	MRL-++	3	—	—	0.5
	4	MRL-++	6	—	—	0.4
	5	MRL-lpr	2	0.2	0	0.1
	6	MRL-lpr	6	0.3	1	0.7
	7	MRL-lpr	6	1.8‡	2	1.4
	8	MRL-lpr	7	6.8‡	4	5.0
	9	MRL-lpr	7	16.9‡	4	3.0
	10	MRL-lpr	7	19.0‡	4	2.0
B	1	C3H/FeJ	3	0.2	—	5.8
	2	C3H/FeJ	5	0.0	—	7.2
	3	C3H/FeJ	6	0.4	—	5.5
	4	MRL-++	4	0.2	—	4.0
	5	MRL-++	7	—	0	7.6
	6	MRL-lpr	2	0.6	1	6.4
	7	MRL-lpr	2	0.3	1	12.1
	8	MRL-lpr	2	0.1	0	4.0
	9	MRL-lpr	3	0.5	1	12.8
	10	MRL-lpr	7	5.1‡	3	22.1
	11	MRL-lpr	7	20.2‡	4	27.6
	12	MRL-lpr	9	2.3‡	2	22.2

* The Northern blots in Figure 2 (A and B) were analyzed by laser densitometry. Each lane represents one animal.
† 24-hour urinary proteins were determined as described (24); ‡ values >1.2 mg/d are considered pathologic.
§ The degree of renal injury was scored on a scale from 0 to 4 as described in legend to Table 1.
‖ Arbitrary densitometric units of the Northern blots were measured by laser densitometry, and ratios were calculated. Because of different exposure times, densitometric ratios in A cannot be compared with the ratios in B and vice-versa.

RNA Extraction and Northern Analysis

Total RNA from kidney cortex was extracted by the guanidinium isothiocyanate/cesium chloride method,[28] as previously described.[23] Total RNA from both transformed and untransformed proximal tubular cell cultures was isolated using a single-step extraction method,[29] as described elsewhere.[27] RNA (25 μg) was denatured with 1M glyoxal and 50% dimethylsulfoxide, electrophoresed through a 1.5% agarose gel, and transferred onto nylon membranes (Gene Screen, New England Nuclear, Boston, MA). The RNA was baked onto the membrane at 80 C for 4 hours, and was then prehybridized with 1.5×S-SPE, 1% SDS, 0.5% (weight/volume) nonfat skim milk and 0.5 mg/ml salmon sperm DNA for 3 hours at 60 C. The cDNA insert of K4-1.1 clone encoding the murine ICAM-1[20] was isolated by *Eco*RI digestion of the plasmid. A 1.3-kb *Hind*III fragment of a genomic I-A_α^b clone[30] was used to probe for MHC class II genes.[23] Probes were radiolabeled by the random primer method.[31] Blots were hybridized for 16 to 24 hours at 60 C (42 C for Ia and β-actin probes) and washed at room temperature with 2×SSC, 0.1% SDS; 0.5×SSC, 0.1% SDS; and 0.1×SSC, 0.1% SDS. The final stringency wash was at 65 C with 0.1×SSC, 1% SDS for 1 hour. After hybridization the blots were exposed to Kodak X-AR film. All blots were also rehybridized with β-actin to ensure that similar amounts of RNA were loaded for electrophoresis. To quantitate specific increases in steady-state mRNA levels, laser densitometry was performed on the blots and ratios of ICAM-1/β-actin and Ia/β-actin were calculated.

Western Blot

24-hour urine collections from C3H/FeJ, BALB/c and MRL-*lpr* mice were dialyzed against phosphate-buffered saline for 5 hours and concentrated. Cellular extracts of transformed proximal tubular cell cultures (C1) and control cells (Concanavalin A-stimulated [5 μg/ml] C3H/FeJ splenocytes and A2A2 T hybrid cells)[27] were made by lysis with 0.5% NP-40. The urine samples and the extracts were then electrophoresed on an SDS polyacrylamide gradient gel (10% to 20%) under reducing conditions[32] and blotted onto nitrocellulose using standard techniques. Blots were blocked with 3% gelatin and incubated with purified biotinylated anti ICAM-1 MAb YN1/1.7.4. After incubation with streptavidin-conjugated alkaline phosphate, the blots were developed with 0.3 mg/ml p-nitro

Table 3. *Densitometric Analysis of ICAM-1 Gene Expression in NZB/W Kidney**

Blot	Lane	Age (month)	U_p† (mg/d)	Histology§ (0–4)	Relative densitometric unit ratios (×10)‖	
					Ia/actin	ICAM/actin
A	1	1.5	—	0	8.9	1.1
	2	1.5	—	0	9.1	1.1
	3	4	—	1	12.1	1.7
	4	4	—	0	12.1	2.3
	5	4	—	0	10.8	2.9
	6	8	2.5‡	2	26.9	4.4
	7	8	2.5‡	2	48.9	4.1
B	1	1.5	—	0	4.4	2.4
	2	4	—	0	10.3	5.0
	3	5.5	0.1	0	14.4	9.5
	4	8	+++‡	2	15.0	8.1
	5	9	3.0‡	2	13.6	8.6
	6	9	3.4‡	3	21.0	11.3
	7	9	12.1‡	3	21.9	7.2

* The Northern blots in Figure 3 (A and B) were analyzed by laser densitometry. Each lane represents one animal.
† 24-hour urinary proteins were determined by dipstick (from − to ++++) or by the TCA method (24); ‡ values > ++ or >1.2 mg/d are considered pathologic.
§ The degree of renal injury was scored on a scale from 0 to 4 as described in Table 1.
‖ Arbitrary densitometric units ratios, reflecting the intensity of the bands seen on the Northern blot. Because of different exposure times, ratios in blot A cannot be compared with ratios in blot B, and vice-versa, as in Table 2.

blue tetrazolium chloride plus 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt.

Results

Immunoperoxidase Staining for the Presence of ICAM-1

In normal C3H/FeJ and MRL−++ mice, basal ICAM-1 expression is detected in PTs, in interstitial cells, and in the endothelium (Figure 1 and Table 1). The glomerular mesangium stains weakly for ICAM-1, but is occasionally positive in older MRL−++. In autoimmune MRL-*lpr* and NZB/W mice with nephritis and proteinuria, increased ICAM-1 expression is detected in PTs, particularly on the brush border side of the cells. A very positive, diffuse mesangial staining pattern is detected in all glomeruli in nephritic MRL-*lpr* and NZB/W mice. The mononuclear infiltrating cells, which are prominent around the main vessels, also stain strongly for ICAM-1. The endothelium of the large vessels in the medulla is also positive for ICAM-1, and stronger staining is detected in MRL-*lpr* and NZB/W mice with nephritis. Table 1 summarizes these data and compares the expression of ICAM-1 to the MHC class II antigens, which we have previously described in MRL-*lpr* mice.[23] As can be seen, Ia is expressed at high levels in nephritic MRL-*lpr*. Nephritic NZB/W have low levels of Ia in PT. ICAM-1, on the other hand, is constitutively expressed in normal PT, but its expression is increased in nephritic MRL-*lpr* and NZB/W mice. Interestingly, in nephritic animals, ICAM-1 is predominantly expressed on the brush border side of the PT, whereas Ia is expressed on the basolateral side. In MRL-*lpr* and NZB/W mice with renal injury, only a few cells in the mesangium express Ia. In contrast, there is a diffuse mesangial staining for ICAM-1 in these nephritic animals.

Northern Analysis for ICAM-1

Figure 2 shows the Northern blot analysis of ICAM-1 gene expression in normal C3H/FeJ, MRL−++, and autoimmune MRL-*lpr* kidneys, and Figure 3 shows the Northern blot analysis for ICAM-1 and Ia gene expression in NZB/W kidneys. Messenger RNA for ICAM-1 is detected in all mice. However, in nephritic MRL-*lpr* and NZB/W, increased steady-state levels are observed, suggesting increased ICAM-1 gene transcription or increased mRNA stability. Tables 2 and 3 depict the densitometric analyses of the Northern blots probed for ICAM-1 and β-actin. The densitometric ratios of ICAM-1/β-actin are increased two- to fivefold in MRL-*lpr* and NZB/W mice with renal disease when compared with normal or nonproteinuric mice. Nephritic NZB/W have also increased ratios of Ia/β-actin, similar to the increased transcripts for Ia in MRL-*lpr* kidney, which we reported earlier.[23]

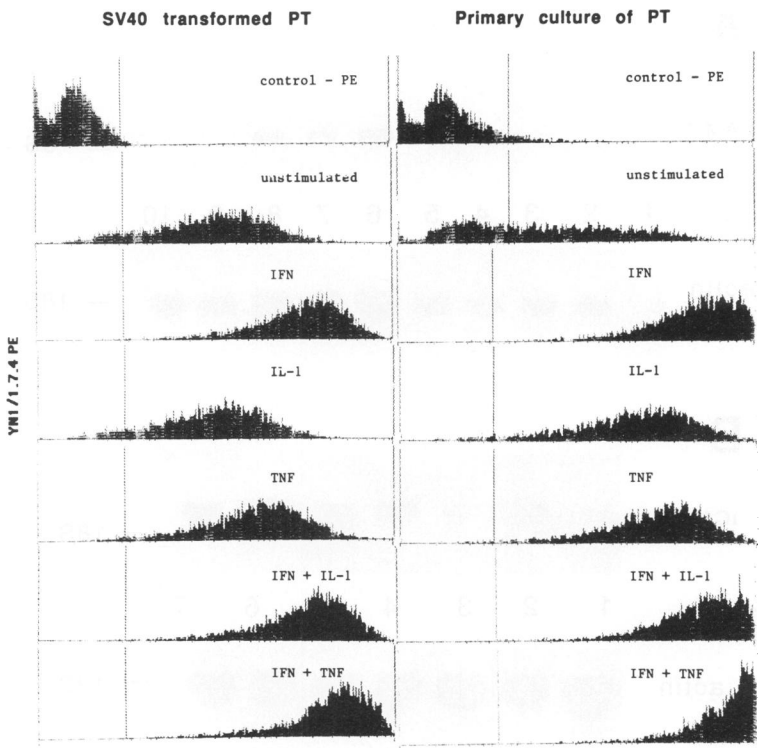

Figure 4. *Flow cytometric analysis of ICAM-1 expression by SV40-transformed PT (left) and by primary cultures of PT (right). Cells were stimulated overnight (17 hours) with rIFN-γ (100 U/ml), rIL-1α (100 ng/ml), rTNF-α (100 ng/ml) or combinations of IFN-γ with TNF or IL-1. After trypsinization, single-cell suspensions of PT were stained with anti-ICAM mAb YN1/1.7.4 (phycoerythrin [PE]-conjugated streptavidin was used as secondary step reagent).*

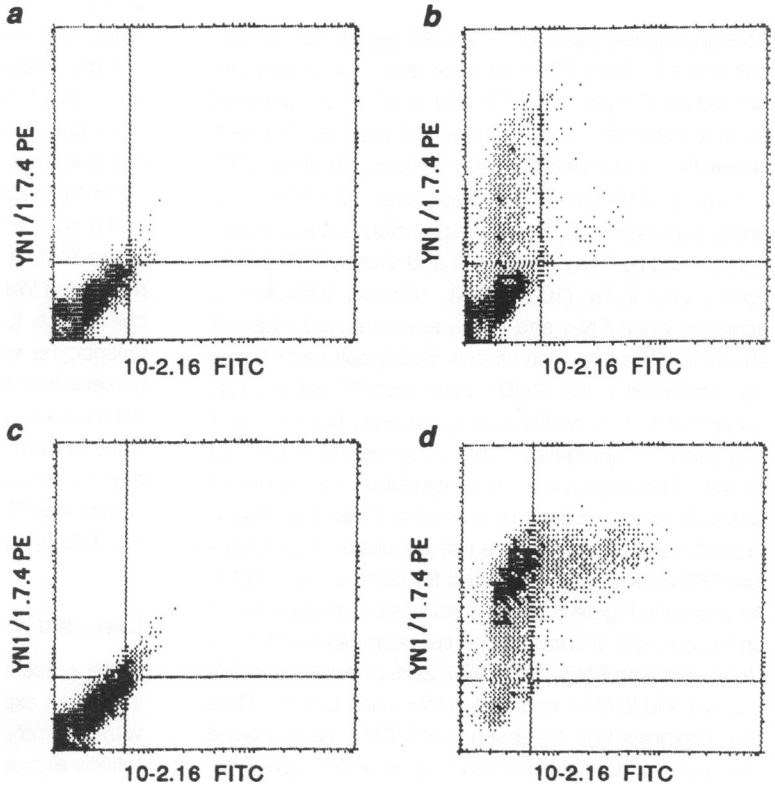

Figure 5. *Flow cytometric analysis of dual-labeled freshly cultured PT from C3H/FeJ mice. Cells were stimulated for 3 days with IFN-γ (100 U/ml) plus rTNF-α (100 ng/ml), trypsinized, and stained with anti-Ia MAb 10-2.16 (x axis) and biotinylated anti-ICAM-1 MAb YN1/1.7.4 (y axis). Phycoerythrin (PE)-conjugated streptavidin and fluorescein-conjugated goat anti-mouse IgG F(ab')$_2$ fragment were used as secondary step reagents.* **a**: *Unstimulated PT, stained without primary antibodies;* **b**: *unstimulated PT, with primary antibodies;* **c**: *IFN-γ + TNF-α stimulated PT, no primary antibodies;* **d**: *stimulated PT, stained with primary antibodies.*

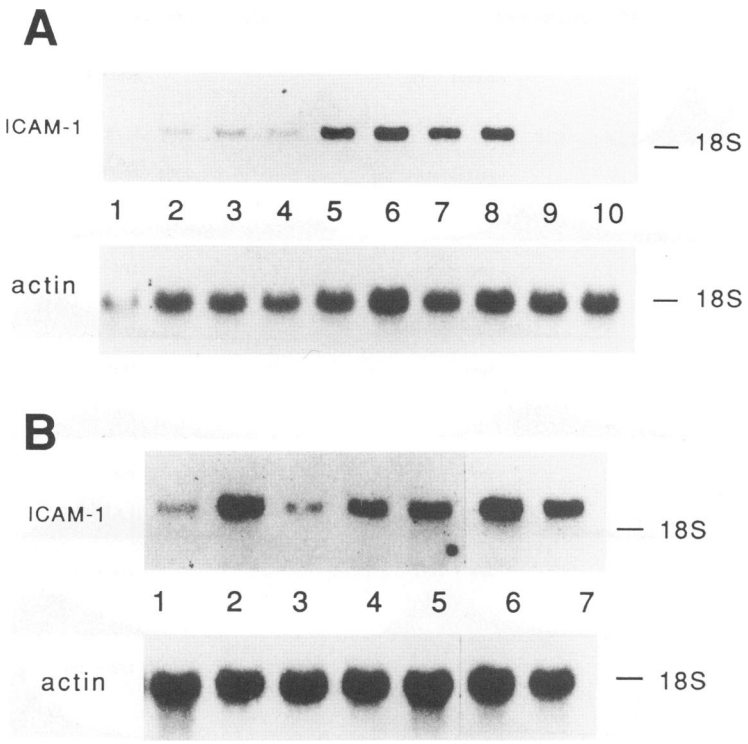

Figure 6. *Northern blot analysis of ICAM-1 gene expression in the transformed PT cell line C1.* **A:** *Transcripts for ICAM-1 are detected in unstimulated C1 (lanes 1–2). Increased levels are detected in response to rIFN-γ (10 U/ml [lanes 3–4], 100 U/ml [lanes 5–6], 1000 U/ml [lanes 7–8]), but not to rIL-4 (1000 U/ml [lanes 9–10]).* **B:** *Lane 1, unstimulated C1 cells; lane 2, rIFN-γ (1000 U/ml); lane 3, rIL-4 (1000 U/ml); lane 4, rTNF-α (100 ng/ml); lane 5, LPS (1 μg/ml); lane 6, rIL-1α (100 ng/ml, Hoffmann LaRoche); lane 7, rIL-1α (100 ng/ml, Pfizer).*

ICAM-1 Expression by Freshly Cultured PT Cells and a PT Cell Line

To study the regulation of PT ICAM-1 expression, freshly cultured PTs from C3H/FeJ mice and a previously described SV40-transformed PT cell line (C1) were analyzed by flow cytometry and Northern blot analysis. Figure 4 shows that unstimulated, freshly cultured PTs and SV40-transformed PTs have a high basal level of ICAM-1 membrane expression. This basal expression is substantially upregulated by IFN-γ (100 U/ml) and also by TNF-α (100 ng/ml) and IL-1α (100 ng/ml). Maximal induction is achieved when IFN-γ and TNF-α are combined. Figure 4 shows that the response seen in freshly cultured PT was also observed in the SV40-transformed PT cell line C1, except for IL-1. In additional experiments, however, IL-1 was found to upregulate ICAM-1 expression in C1 (not shown). The response seen to cytokines was apparent within 24 hours. To determine whether ICAM-1 and Ia expression occurs in the same cell population, freshly cultured PT cells were dual stained for both Ia and ICAM-1. As shown in Figure 5, there is no basal expression of Ia on PT, but 36% of unstimulated cells express ICAM-1. On stimulation with IFN-γ and TNF-α, 26% of cells become Ia positive and ICAM-1 expression increases to 81%. Cells do not express Ia in the absence of ICAM-1, and cytokine stimulation upregulates both molecules on the same population of cells.

The clonal transformed PT cell line C1 was then used to study ICAM-1 gene expression. Figure 6 shows the Northern blot analysis of unstimulated and cytokine-stimulated C1. Transcripts for ICAM-1 are detected in unstimulated cells and are increased after stimulation with IFN-γ in a dose-dependent manner. In addition, IL-1α, TNF-α, and LPS all increase the steady-state levels of ICAM-1 transcripts. IL-4, a known Ia-inducing cytokine for B cells that does not induce Ia on PT, also does not increase ICAM-1 transcript levels.

To compare the ICAM-1 molecule expressed by PT to the ICAM-1 molecule produced by immune cells, we performed Western blot analysis. By flow cytometry, the cell-surface ICAM-1 molecule is detected in activated splenocytes and A2A2 T hybrid cells (not shown). As can be seen from Figure 7, a 90-kD band is detected by MAb YN1/1.7.4 in cellular extracts from PT (C1) and in cellular extracts from Con A-activated C3H/FeJ splenocytes (3-day culture) and the T hybrid cells A2A2. Figure 7 also shows that ICAM-1 is not detected in the urine of C3H/FeJ, BALB/c, and MRL-*lpr* mice.

Discussion

In the present study we describe, for the first time, the increased expression of ICAM-1 in the kidneys of mice with autoimmune murine lupus nephritis. ICAM-1 is constitutively expressed in PTs and in the endothelium of large vessels of normal kidneys. However, ICAM-1 expression is substantially increased on the brush border of PTs, the

endothelium, and in the mesangium in nephritic MRL-*lpr* and NZB/W kidneys. Increased cell-surface expression is paralleled by increased levels of ICAM-1 transcripts in MRL-*lpr* and NZB/W mouse kidneys.

Previously we have reported that nephritic MRL-*lpr* produce substantial amounts of IL-1 and TNF in the kidney.[24,25] Similar increases also occur in the renal cortex of NZB/W mice.[33] Because both IL-1 and TNF can upregulate ICAM-1 in various cell types,[6,7,14–17,19] the increase of ICAM-1 in MRL-*lpr* and NZB/W kidney is likely to be caused by a local release of these inflammatory cytokines. Interestingly, in PT ICAM-1 is induced by all three cytokines IFN-γ, IL-1, and TNF-α, whereas only IFN-γ induces Ia antigens.[27] Therefore, although ICAM-1 and Ia can be coexpressed on the same cell, these molecules are regulated differently.

The increased expression of ICAM-1 by PT in lupus nephritis is a novel finding, although ICAM-1 expression by renal tubular epithelium has been noted in one case of a patient with renal cell carcinoma who had HLA–DR-positive tubules.[11] Of particular interest is its localization to the brush border diametrically opposite to the MHC class II antigens, which are exclusively localized on the basolateral membrane.[34] ICAM-1 is an accessory molecule involved in antigen-presenting function.[2] We have previously reported that PTs have antigen-presenting capacity.[27] Interaction of immune cells with PT through ICAM-1/LFA-1 binding would require basolateral ICAM-1 expression. Although we can not preclude the presence of ICAM-1 on the basolateral membrane of the PT and therefore a role in antigen presentation, its preferential brush border localization in nephritic mice may indicate another role for ICAM-1. Recently it has been shown that ICAM-1 is a receptor for rhinoviruses.[35,36] Because the proximal tubular epithelium is the major reabsorption site of the glomerular filtrate, it is possible that brush border ICAM-1 may act as a nonspecific recognition structure for filtered molecules such as foreign viral proteins or other molecules, thereby contributing to the clearance of foreign or autoantigens.

Because the glomerular mesangium also expresses substantial amounts of ICAM-1 in MRL-*lpr* and NZB/W mice, ICAM-1 could be shed and filtered by a leaky glomerular barrier and then reabsorbed by PTs. However, we do not detect ICAM-1 in the urine of MRL-*lpr* mice, although ICAM-1 may be entirely reabsorbed by the tubules. *In vitro*, however, PTs have the capacity to produce ICAM-1 because cultured PT express ICAM-1 mRNA constitutively, and upregulation is detected in response to cytokines. Thus PTs could increase ICAM-1 gene expression in response to the locally produced cytokines *in vivo*. We speculate that under inflammatory conditions ICAM-1 may have a role in antigen nonspecific recognition by PTs, as well as antigen-specific responses.

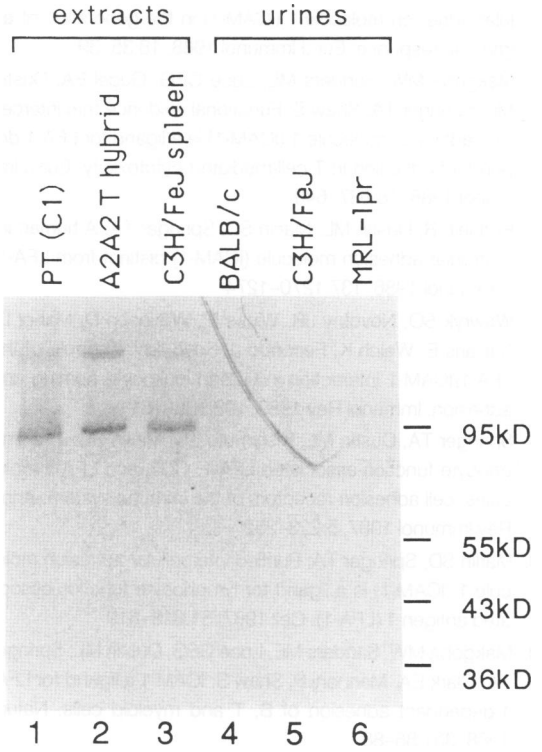

Figure 7. *Western blot for ICAM-1 in cellular extracts from SV40-transformed PT cells, C1 (lane 1), A2A2 T hybrid cells (lane 2), and ConA-activated C3H/FeJ splenocytes (lane 3). A 90-kd band is detected in all cells. ICAM-1 is not detected in the urine of BALB/c (lane 4), C3H/FeJ (lane 5) or MRL-lpr mice (lane 6).*

In summary, increased ICAM-1 transcripts and surface protein are detected in the kidney of MRL-*lpr* and NZB/W mice with lupus nephritis. The glomerular mesangium and the PT are the main sites of increased ICAM-1 expression. In cultured PTs, ICAM-1 is stimulated by inflammatory cytokines such as IFN-γ, IL-1, and TNF. Thus locally produced cytokines may upregulate ICAM-1 *in vivo* in lupus nephritis and promote the autoimmune inflammatory process. The peculiar brush border location of ICAM-1 on PTs suggests that this adhesion molecule plays a novel role.

References

1. Boyd AW, Wawryk SO, Burns GF, Fecondo JV: Intercellular adhesion molecule 1 (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. Proc Natl Acad Sci USA 1988, 85:3095–3099
2. Altmann DM, Hogg N, Trowsdale J, Wilkinson D: Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. Nature 1989, 338: 512–514
3. Dougherty GJ, Hogg N: The role of monocyte lymphocyte function-associated antigen 1 (LFA-1) in accessory cell function. Eur J Immunol 1987, 17:943–947
4. Dougherty GJ, Murdoch S, Hogg N: The function of intercel-

lular adhesion molecule-1 (ICAM-1) in the generation of an immune response. Eur J Immunol 1988, 18:35–39
5. Makgoba MW, Sanders ME, Luce GEG, Gugel EA, Dustin ML, Springer TA, Shaw S: Functional evidence that intercellular adhesion molecule-1 (ICAM-1) ia a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. Eur J Immunol 1988, 18:637–640
6. Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 1986, 137:1270–1274
7. Wawryk SO, Novotny JR, Wicks IP, Wilkinson D, Maher D, Salvaris E, Welch K, Fecondo J, Boyd AW: The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion. Immunol Rev 1989, 108:135–161
8. Springer TA, Dustin ML, Kishimoto TK, Marlin SD: The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. Annu Rev Immunol 1987, 5:223–252
9. Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 1987, 51:813–819
10. Makgoba MW, Sanders ME, Luce GEG, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S: ICAM-1 a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature 1988, 331:86–88
11. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL 1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 1986, 137:245–254
12. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA: Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene family. Cell 1988, 52:925–933
13. Simmons D, Makgoba MW, Seed B: ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature 1988, 331:624–627
14. Pober JS, Gimbrone MA, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 1986, 137:1893–1896
15. Dustin ML, Springer TA: Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 1988, 107:321–331
16. Dustin ML, Singer KH, Tuck DT, Springer TA: Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon γ and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med 1988, 167:1323–1340
17. Griffiths CEM, Voorhees JJ, Nickoloff BJ: Gamma interferon induces different keratinocyte cellular patterns of expression of HLA-DR and DQ and intercellular adhesion molecule-1 (ICAM-1) antigens. Br J Dermatol 1989, 120:1–7
18. Mentzer SJ, Rothlein R, Springer TA, Faller DV: Intercellular adhesion molecule-1 (ICAM-1) is involved in the cytolytic T lymphocyte interaction with a human synovial cell line. J Cell Physiol 1988, 137:173–178
19. Temponi M, Romanco G, D'Urso CM, Wang Z, Kekish U, Ferrone S: Profile of intercellular adhesion molecule-1 (ICAM-1) synthesized by human melanoma cell lines. Sem Oncol 1988, 15:595–607
20. Horley KJ, Carpenito C, Baker B, Takei F: Molecular cloning of murine intercellular adhesion molecule (ICAM-1). EMBO J 1989, 8:2889–2896
21. Siu G, Kuhlman P, Brian A: Isolation and characterization of the murine ICAM-1 gene. J Cell Biol 1989, 107:787a (abstract)
22. Takei F: Inhibition of mixed lymphocyte response by a rat monoclonal antibody to a novel murine lymphocyte activation antigen (MALA-2). J Immunol. 1985, 134:1403–1407
23. Wuthrich RP, Yui MA, Mazoujian G, Nabavi N, Glimcher LH, Kelley VE: Enhanced MHC class II expression in renal proximal tubules precedes loss of renal function in MRL/*lpr* mice with lupus nephritis. Am J Pathol 1989, 134:45–51
24. Boswell JM, Yui MA, Endres S, Burt DW, Kelley VE: Novel and enhanced IL-1 gene expression in autoimmune mice with lupus. J Immunol 1988, 141:118–124
25. Boswell JM, Yui MA, Burt DW, Kelley VE: Increased tumor necrosis factor and interleukin-1β gene expression in the kidneys of mice with lupus nephritis. J Immunol 1988, 141: 3050–3054
26. Tepper RI, Pattengale PK, Leder P: Murine interleukin-4 displays potent anti-tumor activity in vivo. Cell 1989, 57:503–512
27. Wuthrich RP, Glimcher LH, Yui MA, Jevnikar AM, Dumas SE, Kelley VE: Antigen-presenting function and tumor necrosis factor expression by murine renal proximal tubular cell lines. Kidney Int 1990 (In press)
28. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979, 18:5294–5299
29. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987, 162:156–159
30. Ben-Nun A, Choi E, McIntyre KR, Leeman SA, McKean DJ, Seidman JG, Glimcher LH: DNA-mediated transfer of major histocompatibility class II I-A^b and I-A^{bm12} genes into B lymphoma cells: molecular and functional analysis of introduced antigens. J Immunol 1985, 135:1456–1464
31. Feinberg AP, Vogelstein B: A technique for radiolabelling DNA restriction endonuclease fragments to high specificity. Anal Biochem 1983, 132:6–8
32. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227: 680–685
33. Brennan DC, Yui MA, Wuthrich RP, Kelley VE: TNF and IL-1 in NZB/W mice: enhanced gene expression and acceleration of renal injury. J Immunol 1989, 143:3470–3475
34. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS: Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by gamma interferon. J Exp Med 1985, 162:1645–1664
35. Greve JM, Davis G, Meyer AM, Forte CP, Yost SC, Marlor CW, Kamarck ME, McClelland A: The major human rhinovirus receptor is ICAM-1. Cell 1989, 56:839–847
36. Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA: A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell 1989, 56:849–853