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Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites

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Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. To investigate the capacity of lupus autoAb to produce glomerular immune deposits (ID) and nephritis, 24 murine monoclonal (m) anti-DNA antibodies (Ab), derived from either MRL-lpr/lpr, SNF1 or NZB lupus-prone mice and selected based on properties shared with nephritogenic Ig, were administered i.p. (as hybridomas) and i.v. (as purified Ig) to normal mice; at least four mice/mAb were evaluated. Three general patterns of immune deposit formation (IDF) were observed: extracellular ID within glomeruli (± blood vessels, $N = 8$); intranuclear ID ($N = 5$); or minimal or no ID ($N = 11$). The four MRL m anti-DNA Ab that produced significant extracellular ID demonstrated different disease profiles including: (a) mesangial and subendothelial ID with anti-basement membrane staining, associated with proliferative glomerulonephritis, PMN infiltration, and proteinuria; (b) diffuse fine granular mesangial and extraglomerular vascular ID, associated with proliferative glomerulonephritis and proteinuria; (c) dense intramembranous ID and intraluminal ID, associated with capillary wall thickening, mesangial interposition and expansion, aneurysmal dilatation and intraluminal occlusion of glomerular capillary loops, and heavy proteinuria; and (d) mesangial and extraglomerular vascular ID, associated with mild segmental mesangial expansion, without proteinuria. These MRL mAb were derived from four different mice, and they had variable pIs and isotypes. They all cross reacted with multiple autoantigens (autoAg), however, their autoAg binding profiles were distinguishable. Among the SNF1 derived mAb, four produced histologically and clinically indistinguishable disease characterized by diffuse mesangial and capillary wall ID, associated with cellular proliferation/infiltration and proteinuria. Three of the four mAb were derived from the same mouse and were clonally related; they were: IgG2b with SWR allotype, relatively cationic, highly cross reactive with similar Ag binding patterns, idiotypically related and encoded by identical V_H and nearly identical V_L sequences. We conclude that both the capacity of lupus autoAb to form ID and the location of IDF are dependent on properties unique to individual Ig. The results also indicate that the Ag binding region of the autoAb is influential in this process, and they suggest that multiple Ab-Ag interactions contribute to IDF in individuals with lupus nephritis. Furthermore, these observations raise the possibility that the pathologic and clinical abnormalities resulting from these interactions are influenced by the location of IDF, and that the dominant interaction, in a given individual, may be highly influential in the phenotypic expression of nephritis.

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It is generally appreciated that anti-DNA antibodies (Ab) are important contributors to glomerular immune deposit formation in individuals with systemic lupus erythematosus [1–3]. However, the severity of nephritis is not necessarily related to the level of circulating anti-DNA Ab. Many lupus patients with high serum anti-DNA Ab titers do not have severe disease, and conversely, patients with severe disease may have low or barely detectable circulating Ab levels. Furthermore, there are inbred strains of lupus mice with elevated serum levels of anti-DNA Ab that do not develop glomerular disease [1, 4, 5]. Because of these observations, many investigators have concluded that characteristics unique to individual pathogenic anti-DNA Ab influence their capacity to form immune deposits and produce nephritis. To address this issue directly, we and others have examined Ig eluted from mice and patients with active lupus nephritis [2, 4, 6–9]. It has been demonstrated that nephritogenic Ab are predominantly IgG, and they fix complement. Although nephritogenic Ig are more cationic than their serum counterparts in most murine lupus strains, anionic Ig are also prominent, and in some strains the presence of cationic autoantibodies is not sufficient for the development of nephritis [3, 4, 6, 9]. Nephritogenic Ig are enriched for anti-DNA and other autoAb activity (compared to serum), and both components (anti-DNA Ab and other Ig) cross react extensively with a variety of non-nucleic acid autoAg [6, 7]. Additionally, a large fraction of nephritogenic Ig (within a given strain) share idiotypic markers [6, 9, 10]. Collectively, these latter observations indicate that properties unique to the Ag binding regions of pathogenic autoAb may be influential in the initiation of immune deposit formation. In this regard, we and others observed that a subgroup of anti-DNA Ab cross reacted with intrinsic glomerular Ag, in vitro, such as cell surface proteins and basement membrane constituents [11–18]. Furthermore, some anti-DNA Ab produced glomerular immune deposits after administration to normal animals, by cross reacting directly with intrinsic glomerular Ag in vivo [14, 19].

The present studies were undertaken to expand these observations. Our aim was to define a panel of monoclonal (m) autoAb that could both produce immune deposits and transfer disease to normal animals. We reasoned that by doing so, we

could, in turn, more precisely identify the target ligands and determine the relevant V gene sequences of pathogenic autoAb.

Methods

Animals

(AKRxDBA/2)F1 and Balb/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) (SWRxNZB)F1 and (NZBxSWR)F1 were bred in our laboratory as previously described [20] and are designated SNF1 mice.

Production and characterization of hybridoma AutoAb

B cell hybridomas and their mAb products were selected from two large panels derived from MRL-lpr/lpr mice [21]; and SNF1 and NZB mice [9, 20, 22]. Sixteen MRL mAb were selected on the basis of Ag binding and charge properties shared with Ig eluted from the kidneys of MRL-lpr/lpr mice with active lupus nephritis [6]. (The MRL nephritogenic Ab were predominantly IgG, with cross reactive Ag binding properties and varying charge; they also shared idiotypic properties.) The MRL-lpr/lpr mAb all reacted with ssDNA, and many cross reacted with a variety of self-Ag including: double stranded DNA, rabbit thymus extract (containing the SmRNP complex), an extract of extracellular glomerular proteins [14], cardiolipin, hemoglobin, and/or lipopolysaccharide. Their charge varied with pI's from 5.1 to 8.0.

Eight SNF1 and NZB mAb, previously characterized in vitro, were selected for in vivo analysis [9, 20, 22]. Three SNF1 mAb, 550, 564 and 567, were IgG2b with the SWR allotype, cationic (pI's = 8.28, 8.32, 8.15), had similar Ag binding patterns, and shared idiotypic properties with each other and with nephritogenic Ig. (Ig eluted from SNF1 nephritic kidneys are predominantly cationic IgG, enriched for autoAg binding properties, and they share idiotypic markers, IdLNF1). These three Ig were independently derived hybridomas from a single SNF1 mouse, and molecular analysis indicated that they arose from a single B cell precursor [23, 24]. They had identical V_H region nucleotide sequences, however, their V_K region sequences differed from each other by one to three nucleotide substitutions. Another SNF1 mAb, 548, a cationic (pI = 8.65), IgG3 with the NZB allotype, also shared idiotypic properties with nephritogenic Ig, but it was derived from a separate mouse and used a V_H gene (V_H family 7183) distinct from 550, 564 and 567 (V_H family J558). The mAb 317, an SNF1 derived, cationic (pI = 7.87), IgG2a, with the NZB allotype, shared idiotypic properties with nephritogenic Ig. Three other mAb did not share the nephritogenic idiotype IdLNF1: 532 (an SNF1 derived, IgG2b, pI = 7.14, SWR allotype), 205 (an NZB derived IgG2a, pI = 6.39) and 524 (an SNF1 derived, IgG2b, pI = 7.14, SWR allotype).

mAb were purified from hybridoma supernatants by affinity chromatography [14]. The Ag binding properties of the MRL mAb were examined by both direct binding and competitive inhibition in ELISAs using the following purified Ag: ssDNA, dsDNA, SmRNP and glomerular extract [6, 14, 21]. Isotype determination and isoelectric focusing of Ig were performed using previously described methods [6, 14]. V_H gene family usage was determined by a modification of the Manser and Gefter technique [21, 25] or by hybridization to Northern blots [26].

Administration of murine hybridomas and purified mAb to normal mice

Murine hybridomas. Ten days after pristane-priming, 5×10^6 hybridoma cells were injected into the peritoneum of either four- to six-week-old (AKRxDBA/2)F1 mice (if the hybridoma was derived from an MRL-lpr/lpr mouse) or into four- to six-week-old pre-autoimmune SNF1 mice (if the hybridoma was derived from either an NZB or SNF1 mouse). In parallel, either age-matched, pristane-primed normal mice or PBS-injected, pristane-primed, preautoimmune SNF1 mice were used for comparison. After 7 to 21 days, mice that developed visible ascites and/or tumor growth were tail-bled, and their serum was tested for: anti-DNA Ab activity (ELISA), antinuclear Ab activity (indirect immunofluorescence; IF) [14] and cryoglobulinemia (incubation of serum at 4°C for 72 h). Only those animals that developed both visible ascites (and/or tumors) and serum anti-DNA Ab activity were subjected to further examination. Although the time required to develop ascites varied, mice were always sacrificed three to five days after the onset of visible ascites. Observations were made on at least four animals that developed ascites in each group. Urine protein excretion was measured in the (AKRxDBA/2)F1 using the Bio-Rad Assay (Bio-Rad, Richmond, California, USA) on 24 hour collections obtained in metabolic cages [27]. After urine collection the animals were sacrificed, and blood, ascites, kidneys and other organs were isolated and saved for further study.

Kidney sections from each animal were analyzed by direct IF, and electron microscopy (EM), as previously described [27]. For light microscopy (LM), one entire kidney from each animal was fixed in 10% formalin and embedded in paraffin. The number of nuclei/glomerulus and glomerular size were determined as previously described [28]. Briefly, multiple 4 μm sections through the center of the longitudinal axis of each whole kidney were obtained, and they were stained with hematoxylin and eosin. The cross sectional diameter and the number of nuclei/glomerulus were determined on thirty consecutive glomeruli, using an ocular micrometer. For comparative analysis, the ten largest glomeruli on each slide were utilized. This maneuver increases the probability that measurements were determined on sections through the middle of the glomerulus (as opposed to the poles) and is a more accurate indicator of glomerular volume. Quantitative analyses were performed on animals selected at random from each group. Statistical evaluation of the groups was performed by one-way analysis of variance using nonparametric statistics by Kruskal-Wallis one-way analysis of variance by ranks [29, 30]. Where appropriate, the groups were compared using the Mann-Whitney U test [29, 30].

Isoelectric focusing of Ig deposited within glomeruli was performed by direct elution of Ig from tissue sections and previously described methods [6, 9]. Briefly, 25 μm sections were washed thoroughly with PBS and then applied directly to the gel (1% agarose). After 30 to 60 minutes of electrophoresis, the sections were removed, and electrophoresis was continued for a total of two hours. After transfer to nitrocellulose the Ig were visualized using isotype-specific goat anti-mouse Ig by using either the alkaline phosphatase method previously described or the ECL detection system (Amersham Corporation, Arlington Heights, Illinois, USA). In pilot studies using 25 μm kidney sections derived from normal mice, Ig was not detected by this method, indicating that serum Ig did not influence the results.

Table 1. Summary of glomerular, tubular and vascular immune deposits induced by MRL-lpr/lpr m anti-DNA Ab

mAb #	Immune deposits		
	Glomerular	Vascular	Tubular
H147	linear bm mes	linear	liner
H238	mes capillary wall	small vessels fine granular	none
H221	intraluminal mes/subendo	none	none
H161	mes capillary wall	small vessels intimal	none

Abbreviations are: bm, basement membrane; mes, mesangial; subendo, subendothelial.

Table 2. Quantitative glomerular morphology and 24-hour urine protein excretion in mice injected with MRL anti-DNA antibody producing hybridomas

mAb	Nuclei/ glomerulus (*N*)	Glomerular size *μm* (*N*)	Proteinuria *mg/day* (*N*)
H147	44.3 ± 1.2[b] (3)	107 ± 6.7[a] (3)	1.8 ± 0.4[a] (4)
H238	48.7 ± 3.8[b] (3)	107 ± 2.6[b] (3)	1.9 ± 0.6[a] (4)
H221	32.0 ± 2.7 (4)	87 ± 0.7[b] (4)	3.8 ± 1.2[a] (7)
H161	32.5 ± 3.5 (2)	83 ± 1.5 (2)	1.0 ± 0.2 (3)
Pooled results[c]	27.4 ± 0.8 (13)	80.4 ± 1.3 (13)	0.9 ± 0.1 (8)

(*N*) = number of mice evaluated in each group
[a] $P < 0.05$; [b] $P < 0.01$, compared to pooled results[c]
[c] Pooled results from H73 (# = 4), H32 (# = 6) and H149 (# = 3) mice; they produced ascites and elevated serum anti-DNA levels but did not produce either intranuclear or extracellular immune deposits.

Administration of purified Ig. In separate experiments, 1 to 2 mg of purified MRL-lpr/lpr mAb were injected i.v. to normal four- to six-week-old BALB/c mice that had undergone uninephrectomy two weeks prior to injection. After four to six hours, the animals were sacrificed, and the kidneys were evaluated by direct IF and LM.

Results

Following administration of either hybridomas or their corresponding anti-DNA mAb to normal mice, three general IF patterns were observed: (a) extracellular immune deposits within glomeruli and blood vessels (# = 8); (b) intranuclear Ig deposition within cells of the kidney and other organs (# = 5); and (c) no detectable immune deposits (# = 11). Eight mAb (4 MRL, 4 SNF1) that reproducibly produced extracellular immune deposits within glomeruli ± blood vessels were subjected to further in vivo and in vitro analysis. At least four animals in each group were evaluated (5 for SNF1), and in all cases immune deposits were visualized in each animal within the groups. The pattern of immune deposits and the disease profile were indistinguishable between animals within a group with the exception noted below (H147). Many of the mAb (# = 11) either did not form immune deposits or produced only minimal mesangial deposits that were indistinguishable from those in animals that received only pristane, despite the production of high serum levels of circulating anti-DNA Ab; three are presented for comparative purposes. Every animal used in the study had elevated serum levels of anti-DNA Ab, and in many cases the anti-DNA Ab titers in the animals that did not form significant immune deposits were higher than those that did (not shown). The mAb that produced intranuclear deposits (with no extracellular deposits) are the subject of a separate report (5 mAb: 3 from MRL, 1 SNF, 1 NZB) [28]. For purposes of presentation, the MRL and SNF1 mAb will be considered separately.

The results of the in vivo analyses of the MRL mAb are presented in Figures 1 to 4 and Tables 1 and 2. The location of immune deposits varied with the administered mAb. This was associated with differences in the morphologic and clinical expression of disease; multiple histologic and clinical patterns were observed. Representative observations from each group (mAb) are illustrated. With a few notable exceptions (see below), following i.v. injection the MRL mAb produced immune deposits in a pattern that was indistinguishable from the IF pattern in the mice that received the corresponding hybridomas. However, since the goal of the i.v. studies was to determine the capacity of the purified Ig to form glomerular immune deposits, these animals were sacrificed after four to six hours, and neither histologic nor clinical parameters of nephritis were evaluated. For purposes of clarity, the results for each mAb will be presented separately, however, they are also summarized in Table 1. Representative results from mice that received hybridomas and produced ascites with elevated serum anti-DNA Ab levels but did not form immune deposits, are indicated in Table 2 for comparison.

H147 produced granular mesangial and subendothelial deposits in all animals (Fig. 1). In two of the five mice that received the H147 hybridomas, linear staining along capillary loops, glomerular capsules, tubules and vessels was also observed (Fig. 1). To further evaluate the linear staining pattern, an additional group of five mice was studied: two developed prominent diffuse linear deposits identical to those described above; two had faint or no linear basement membrane staining, and one animal died prior to evaluation. This linear pattern was not found in any of the mice that received purified H147 Ig i.v. Proliferative glomerulonephritis with PMN infiltration, and proteinuria was present in all the H147 hybridoma mice (Table 2). Following elution and IEF of the Ig derived from the kidneys of the mice that received the hybridomas, IgG bands were observed in a pattern identical to that of purified H147; IgM were not visualized.

After administration of hybridomas, H238 produced diffuse fine granular mesangial, capillary wall, and extraglomerular vascular deposits (Fig. 2). An identical pattern was also observed after i.v. injection of H238, however the intensity of staining was less in all locations. All of the hybridoma mice developed mesangial proliferation and expansion, PMN infiltration, and proteinuria (Table 2). IEF of the Ig eluted from the kidneys of the hybridoma mice indicated that the major IgM band was identical to purified H238; an additional minor IgM band was also visualized, but IgG bands were not observed.

Capillary wall and dense intraluminal IgG deposits were

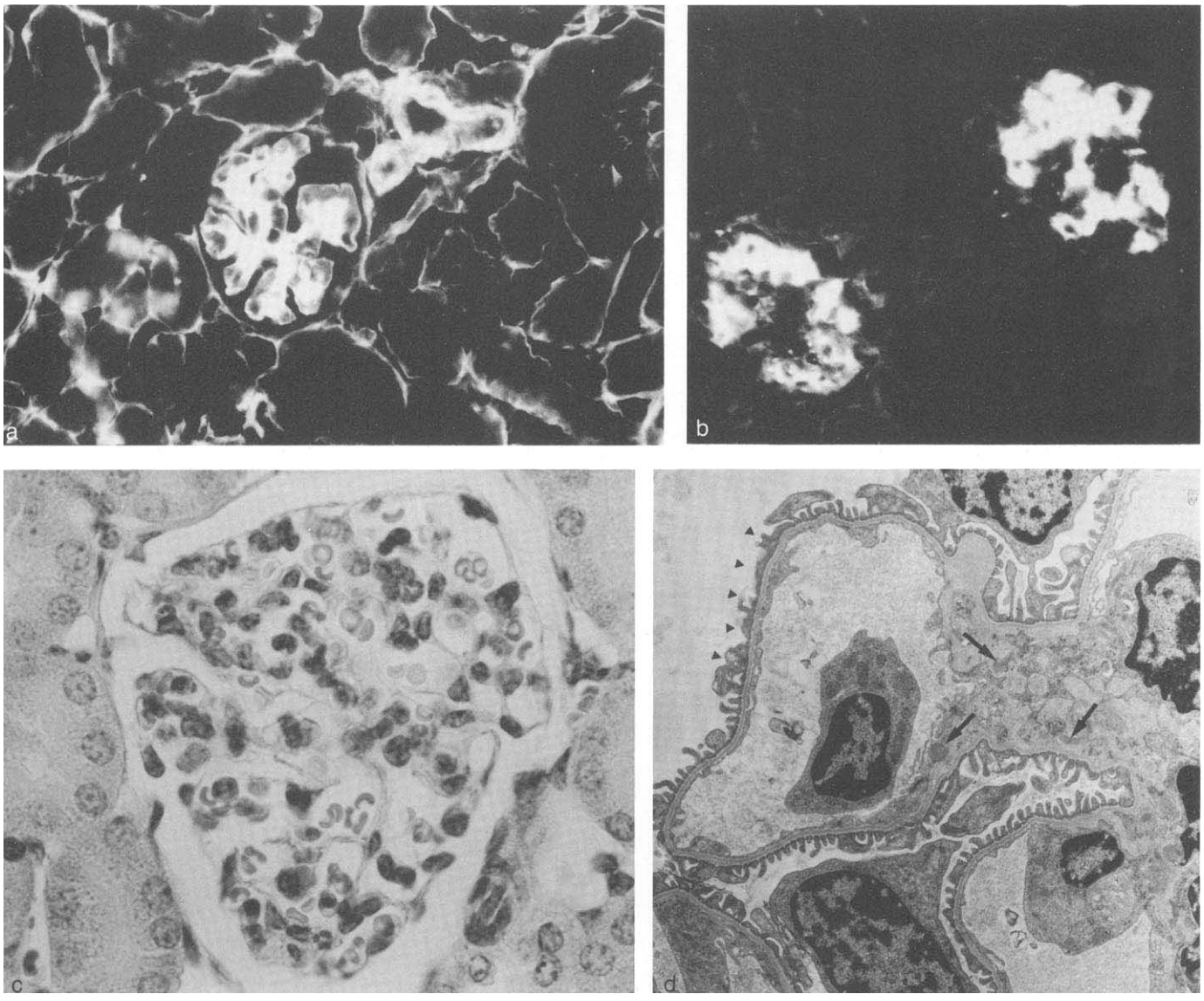

Fig. 1. *Histologic evaluation of kidneys from normal 4- to 6-week old mice following administration of H147.* **(a)** Direct IF of kidney from mouse that received H147 hybridomas: linear deposits of IgG along glomerular capillary loops, glomerular capsules, tubules and extraglomerular vessels; granular IgG deposits were also observed within glomeruli (270×). **(b)** Direct I.F. of kidney from mouse that received affinity purified H147, 4 hours after intravenous injection: IgG within glomeruli, including capillary loops and mesangium; tubular and vascular deposits were not observed (200×). **(c)** L.M. of kidney from mouse that received H147 hybridomas: enlarged glomerulus with cellular proliferation and PMN infiltration (350×). **(d)** EM of kidney from mice that received H147 hybridomas: arrows indicate mesangial deposits; subendothelial deposits were observed more abundantly in other fields. Arrow heads indicate foot process fusion (3500×). Fiber bundles with the ultrastructural appearance of collagen were observed within the mesangium (not shown).

visualized within glomeruli of all mice that received H221 hybridomas (Fig. 3). This was associated with capillary wall thickening, mesangial interposition and expansion, aneurysmal dilatation and intraluminal occlusion of glomerular capillary loops, and heavy proteinuria (Table 2). The intraluminal deposits were reminiscent of immune deposits associated with cryoglobulinemia [31], however, the mice did not have detectable cryoglobulins in their serum. The intraluminal deposits were not visualized following i.v. injection of purified H221. IEF of Ig eluted from the kidneys of the hybridoma mice, demonstrated a single band with a pI of 5.1, and IgG2a was the only isotype detected.

Following administration of either H161 hybridomas or purified H161, mesangial and extraglomerular vascular deposits were observed (Fig. 4). The hybridoma mice developed segmental mesangial expansion and mild mesangial cell proliferation. Glomerular and extraglomerular vascular occlusion were also observed. The level of proteinuria was not significantly different from the control mice. IEF of eluted Ig revealed IgG with a pI of approximately 7.0 (6.8 to 7.2) and the absence of IgM.

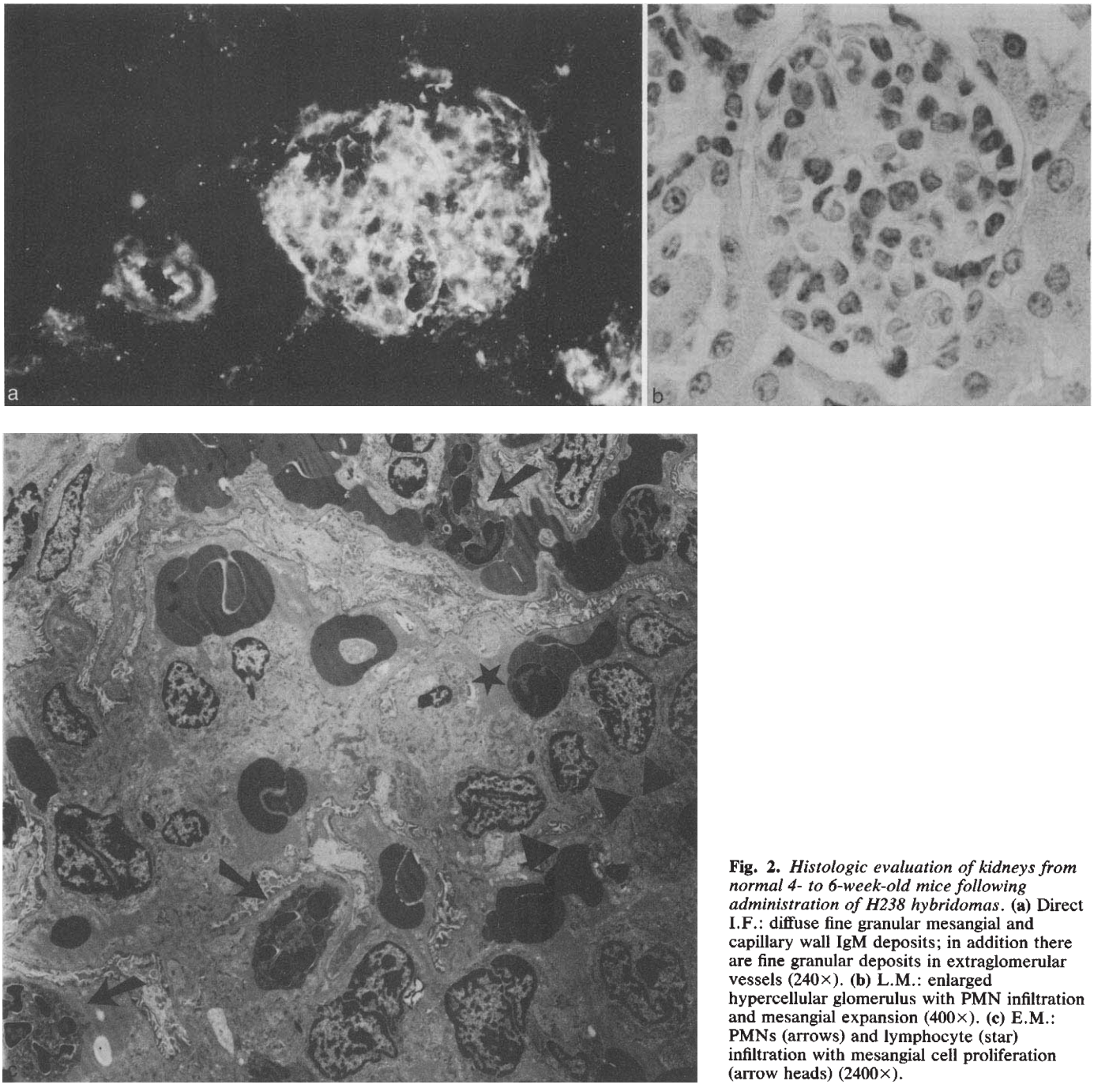

Fig. 2. *Histologic evaluation of kidneys from normal 4- to 6-week-old mice following administration of H238 hybridomas.* **(a)** Direct I.F.: diffuse fine granular mesangial and capillary wall IgM deposits; in addition there are fine granular deposits in extraglomerular vessels (240×). **(b)** L.M.: enlarged hypercellular glomerulus with PMN infiltration and mesangial expansion (400×). **(c)** E.M.: PMNs (arrows) and lymphocyte (star) infiltration with mesangial cell proliferation (arrow heads) (2400×).

Although the mAb were selected because they cross reacted with multiple autoAg, in a manner analogous to Ig eluted from the kidneys of mice with active nephritis, each Ab had distinct profile of Ag binding to the panel of autoAg (Table 3). In addition to the greater reactivity to dsDNA and SmRNP, H221 could be further distinguished from H147 by reactivity to lipopolysaccharide and levan (H147 did not react with these Ag) [21]. The Ag binding specificities of the mAb that formed immune deposits, using this limited group of autoAg, could not be differentiated from the mAb that did not form deposits (data not shown), however, this does not exclude the possibility that the two groups of mAb differ in their ligand binding specificities for other unknown autoAg(s). The Ig subclass, charge, and VH gene use varied among the MRL-derived mAb that formed immune deposits, and it was not possible to distinguish them, by these in vitro properties, from the mAb that did not form deposits.

Three general IF patterns were also observed for the SNF1 mAb. One group, consisting of 550, 564, 567, and 548, produced extracellular glomerular immune deposits; another group produced only intranuclear deposits (317 and 205; [28]); and two

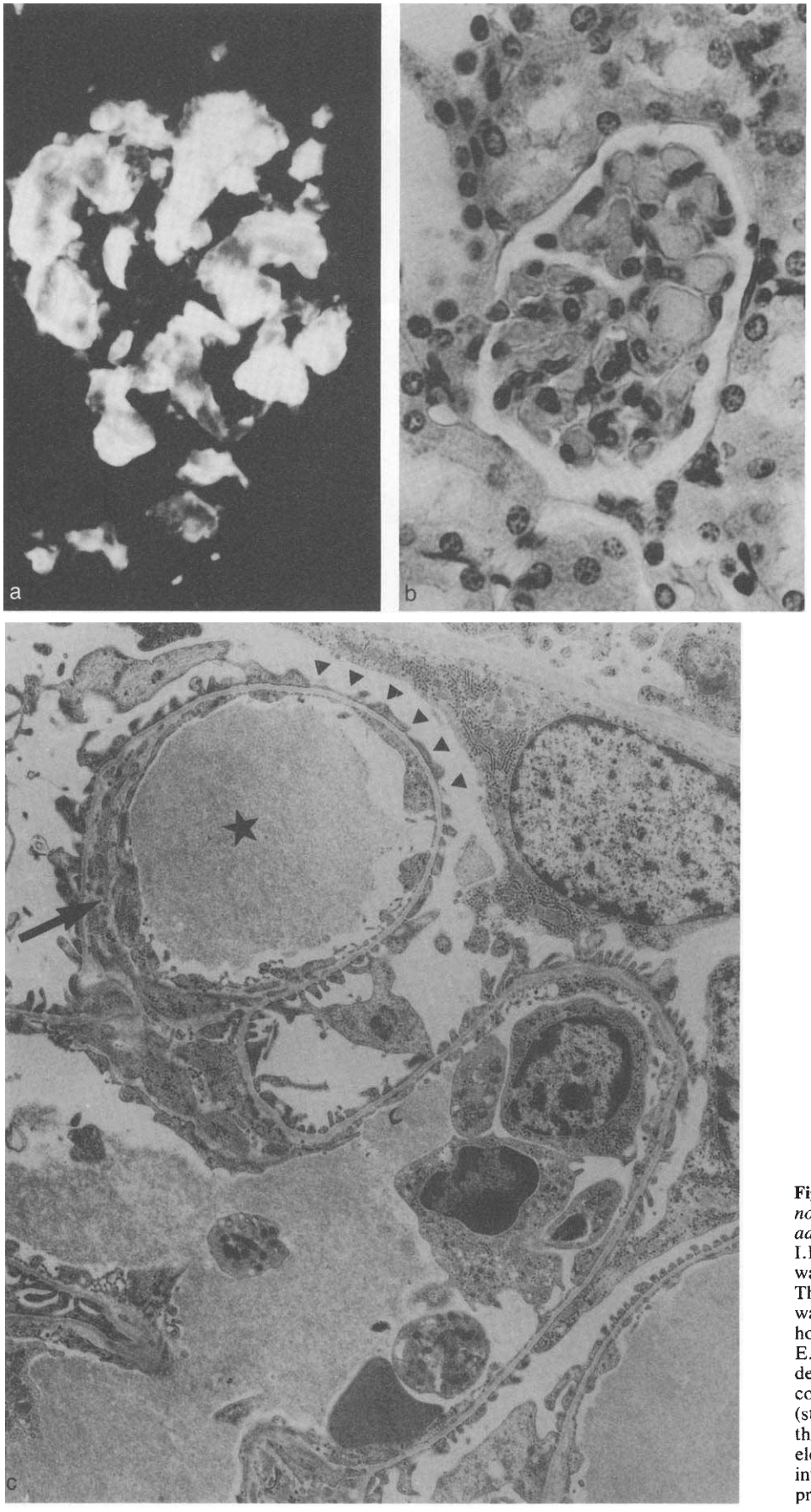

Fig. 3. *Histologic evaluation of kidneys from normal 4- to 6-week-old mice following administration of H221 hybridomas.* **(a)** Direct I.F.: Diffuse dense intraluminal and capillary wall deposits of IgG (300×). **(b)** L.M. Thickened appearance of glomerular capillary walls, capillary loop dilatations with homogeneous intraluminal material (450×). **(c)** E.M.: large amorphous electron dense deposits within distended capillary lumen corresponding to IgG deposits by I.F. above (star); foot process fusion (arrowheads), and thickened capillary walls associated with electron dense deposits, and mesangial interposition. Mesangial expansion is also present (5000×).

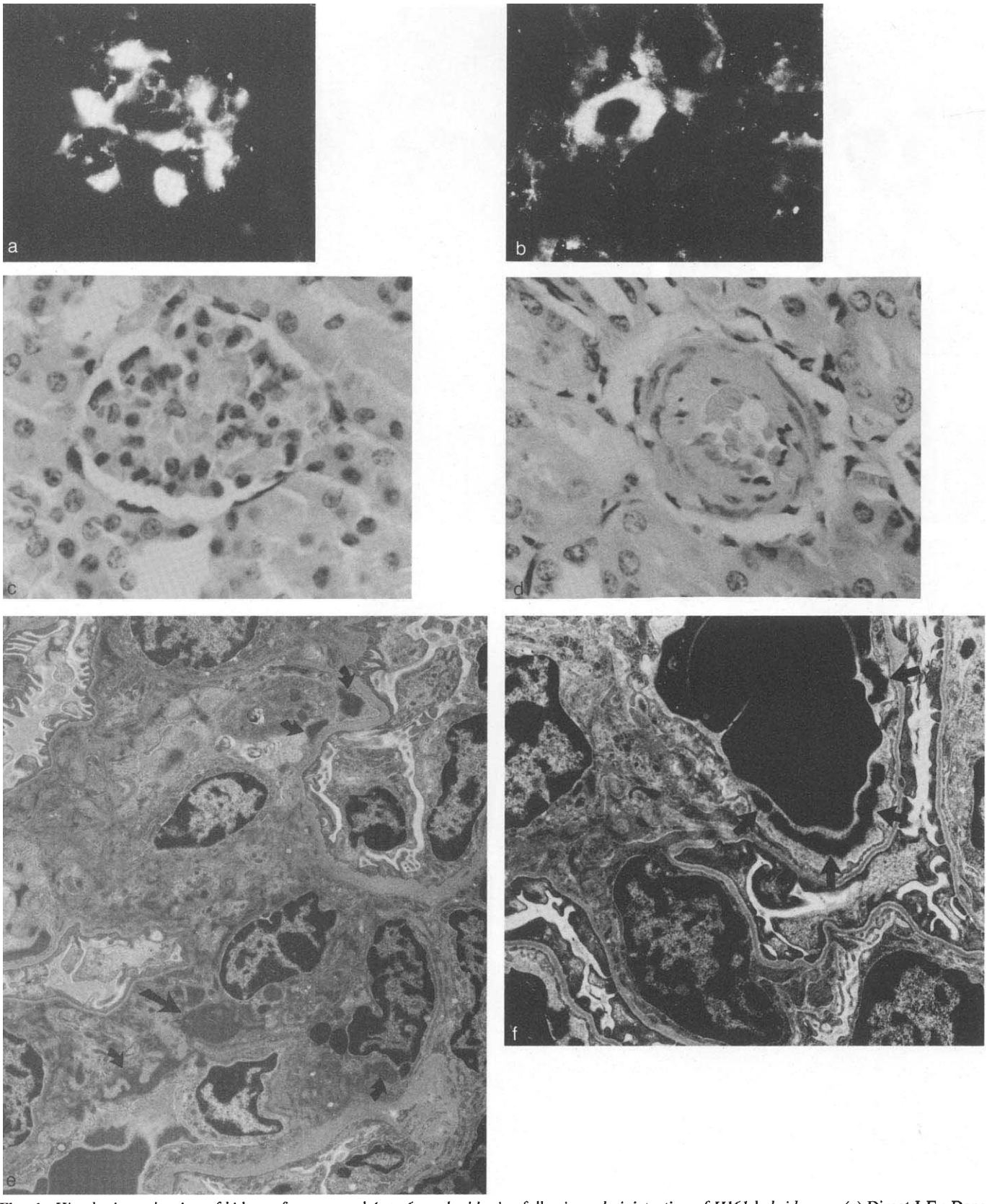

Fig. 4. *Histologic evaluation of kidneys from normal 4- to 6-week-old mice following administration of H161 hybridomas.* **(a)** Direct I.F.: Dense granular mesangial IgG deposits with fine granular deposits of IgG involving some capillary walls (350×); **(b)** Direct I.F.: a vessel outside the glomerulus with granular IgG deposition limited to the intimal surface (350×); **(c)** L.M.: segmental mesangial expansion with borderline hypercellularity (300×); **(d)** L.M.: hyalin thickening of a vessel wall (450×); **(e)** E.M.: numerous electron dense deposits within the mesangium (arrows) (3900×); **(f)** E.M.: dense intraluminal deposits (arrows) corresponding to IF deposits in (a) (6900×).

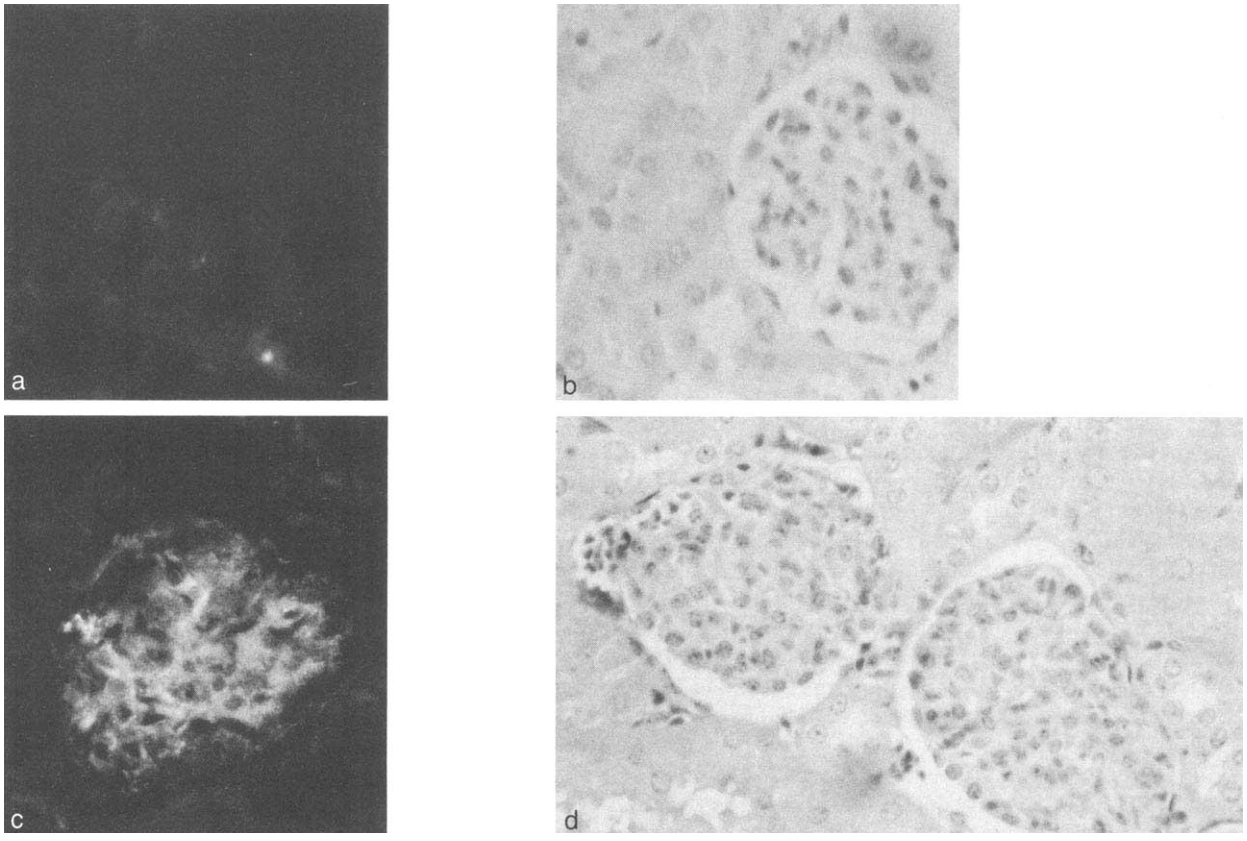

Fig. 5. *Histologic evaluation of kidneys from 4- to 6-week-old SNF1 mice following administration of anti-DNA producing hybridoma.* **(a)** I.F. and **(b)** L.M. from 532 injected mice. This appearance was not significantly different from age-matched PBS-injected, pristane-primed SNF1 mice. **(c)** I.F. and **(d)** L.M. from 567 injected mice. (c) Diffuse mesangial and capillary wall deposits involved all glomeruli. (L.M. and I.F. are 300×) (d) Glomerular hypercellularity with PMN infiltration. The glomerular mAb 560, 564, and 548 were indistinguishable from 567.

mAb did not produce immune deposits (524 and 532). In contrast to the MRL mice, the morphologic and clinical expression of disease among the individual mice that received SNF1 mAb, 550, 564, 567, and 548, were indistinguishable by IF, LM and EM. A representative example is illustrated in Figure 5; the disease was characterized by diffuse mesangial and capillary wall deposits, mononuclear cellular proliferation/infiltration and PMN infiltration. These abnormalities were observed in all animals within each of the groups (5 mice/mAb).

Discussion

In these studies m anti-DNA Ab derived from lupus mice produced glomerular and vascular immune deposits in normal and pre-autoimmune mice following adoptive transfer. Each immune-deposit forming Ab consistently produced the same abnormalities in individual animals, however the location of immune deposits varied among m Ab. In each case, the sole or principal Ig within the lesions was the administered Ig. Immune deposit formation, *per se*, was not purely a quantitative phenomenon, because many mice had elevated serum anti-DNA Ab levels without immune deposits, and immune deposits were observed after i.v. injection of relatively small quantities of Ab. Furthermore, the observed differences in the location of immune deposits with individual Ab were not due purely to quantitative differences in circulating Ab levels for similar reasons. It is likely, therefore, that the physical and immunologic properties of Ig influenced both their capacity to form immune deposits and the site of immune deposit formation within glomeruli and blood vessels. These properties as well as potential factors contributed by the experimental model warrant further discussion.

The capacity of a subgroup of anti-DNA Ab to form immune deposits, whereas other isotype-matched autoAb did not, is consistent with an influential role of the Ag binding region in this process. This was not unexpected, because we had previously observed that immune deposit formation was not produced by all anti-DNA Ab on passive transfer [14]. The influence of the Ag binding region was also suggested by our previous analysis of Ig eluted from lupus kidneys: autoreactivity, cross-reactive autoAg binding specificities and shared idiotypes distinguished nephritic Ig from their serum counterparts [6]. The variations in the location of immune deposits with individual mAb, observed in the present study, provide new information relevant to understanding the mechanisms of these events. The results suggest that the Ag binding region may also influence the site of immune deposition. Variability in the Ag binding properties among the individual MRL m anti-DNA Ab is consistent with this conclusion, and the similarity of Ag binding properties of the SNF1 mAb that produce indistinguishable patterns of disease also supports this viewpoint (see

Table 3. In vitro characteristics of MRL-lpr/lpr immune deposit forming mAb

mAb #	Age *months*	Isotype	pI	V_H gene family	Antigen binding properties[a] ssDNA	dsDNA	SmRNP	GE
H147	3	IgG2a	8.0	7183	3+	2+	1+	2+
H238	1	IgM	7.5	J558	1+	–	3+	2+
H221	6	IgG2a	5.1	J558	3+	3+	2+	2+
H161	6	IgG3	7.0	J558	2+	0	0	0

[a] As determined in ELISA; scale = minimum concentration of mAb that produced OD405 >0.500, when positive control mAb reading was between 1.0–1.2 and negative control mAb <0.050; 3+ = <0.1 μg/ml; 2+ = 0.1–1.0 μg/ml; 1+ = 1–10 μg/ml; 0 = >10 μg/ml. Age indicates age of mouse from which hybridoma was derived.

below). The influence of the antigen binding region among the MRL mAb is also indicated by differences in V_H gene family use (indicating less that 80% nucleotide sequence homology between their V_H genes) among H147 and H221. These Ig had markedly different pIs and distinguishable antigen binding profiles (Table 3 and **Results**) despite constant region identity (IgG2a). Collectively these observations raise the possibility that there may be subsets of pathogenic lupus autoAb, with distinct cross-reactive Ag binding properties, that are each responsible for immune deposit formation at distinct locations within the glomerulus.

One or more of the previously defined mechanisms of immune deposit formation for lupus autoAb may be operative in these individual circumstances, including cross-reactivity of autoAb with intrinsic glomerular antigens, in situ formation of complexes of anti-DNA Ab with autoAg (that have an affinity for specific sites within the glomerulus), or passive trapping of circulating immune complexes [11–14, 17, 19, 32–34]. Indeed, differences in either the site and/or mechanism of immune deposit formation, determined by the Ag binding region, could contribute to the observed experimental differences [35]. These possibilities clearly deserve further study. In this regard, further investigation of H238 and H161, including comparison of their V region sequences and determination of the mechanisms of immune deposit formation, will be of considerable interest, because these MRL mAb both produced mesangial and subendothelial immune deposits.

Although the morphologic pattern of disease varied with the MRL-derived antibodies, the SNF1 mAb produced mesangial and capillary wall deposits that were indistinguishable by IF. These similarities may be explained by nearly identical structure of three of the four mAb: 550, 564, and 567 have identical H chain V region sequences (548 has not been sequenced), their V_K sequences differ from each other by only one to three nucleotides, and their in vitro properties are indistinguishable [23, 24]. Since cationic amino acid residues within CDR 1 and CDR 2 distinguish this group of mAb, we suggest that these residues determine the capacity of this group of pathogenic autoAb to form immune deposits. Furthermore, since a large fraction of nephritogenic Ab in this strain share idiotype, charge and Ag binding properties with this group of autoAb [9, 22], it is likely that this amino acid sequence motif may strongly influence disease expression in SNF1 mice. The previous observation that 548 shares idiotypic properties with 550, 564, 567 and Ig eluted from the kidneys of SNF1 mice is also consistent with this conclusion. These results also reinforce the reproducibility of the experimental model and its dependence on individual properties of the mAb: three independent hybridomas each secreting highly related but distinct Ig, produced pathologically indistinguishable disease.

The potentiating role of hybridomas in immune deposit formation deserves additional consideration. We and others have utilized this method to examine the capacity of individual autoAb to form immune deposits and transfer disease [14, 31, 36]. This approach has some practical and theoretical advantages: (i) it provides high levels of circulating Ig for prolonged periods, a situation that approximates circulating levels of autoAb in the serum of individuals with active lupus; (ii) the intraperitoneal inflammation that accompanies this manipulation may mimic the inflammatory milieu associated with disease activity; and (iii) the presence of elevated cytokine levels and other inflammatory mediators could potentially facilitate immune deposit formation either by release of circulating autoantigens or through expression of neoantigens locally within the kidney. Nevertheless, the extent to which these conditions exist in individuals with lupus requires confirmation. In the present studies, the location of the immune deposits were similar following the i.p. and i.v. maneuvers in most cases, however, there were two circumstances where the location of immune deposits were unique to hybridoma administration: the linear basement membrane deposits observed in some of the H147 hybridoma mice and the intraluminal deposits associated with all mice that received the H221 hybridomas. These differences may have been related to either quantitative differences in the level of circulating Ab or exposure of neoantigens induced by the hybridomas. Additional studies are required to distinguish between these possibilities. It is highly unlikely that these differences were due to a secondary Ab response (that is, rheumatoid factor or anti-idiotypic), because additional bands would have been expected by IEF of Ig derived from the kidney sections. Furthermore, the hybridoma milieu is known to suppress production of host Ig [37].

The pathologic and clinical expression of disease varied with the administered MRL mAb, however, individual mAb always produced the same abnormalities. It is unlikely that these differences were due to the capacity of individual Ig to recruit other Ig (that is, rheumatoid factors, anti-idiotypes) for reasons discussed previously. The differences in disease expression, therefore, were most likely related to either quantitative differences in Ig deposition, the pro-inflammatory properties of individual mAb and/or the location of immune deposit formation. Each of these factors is known to influence the morphologic and clinical expression of disease [35]. Additional studies with each mAb will be necessary to distinguish between them. Nevertheless, these results indicate that individual anti-DNA Ab can be pathogenic. Furthermore, they raise the possibility that the Ag binding region of individual autoAg, by determining the location of immune deposit formation, directly influences the nature and extent of disease expression.

The influence of Ag binding region on pathogenicity is also suggested by the recent observations of Reininger and coworkers [31]. They compared the pathogenicity of an MRL-lpr/lpr-derived IgG3k rheumatoid factor with cryoglobulin activity, termed 6-19, to a hybrid mAb that consisted of the 6-19 heavy chain and an unrelated lamda light chain. The original autoAb,

6-19, produced vasculitis and glomerulonephritis, however the recombinant protein only induced glomerulonephritis. Since the pathologic profile of the mAb was altered (and presumably the Fc region was not changed), the results emphasize the contribution of different V region segments to the pathogenicity of an individual Ig.

The present observations have several important implications. They suggest that both the site of immune deposit formation and the resulting pathologic and clinical abnormalities may be dependent on properties unique to subsets of anti-DNA Ab. In this regard, the lupus nephritis phenotype may be dependent not only on the quantity and pro-inflammatory properties of autoAb, but also on the Ag binding properties of the pathogenic Ig that predominate in a given individual. These results provide a rationale, therefore, for the investigation of V region sequences associated with pathogenic autoAb production. Moreover, the results raise the possibilities that multiple Ab-ligand interactions participate in immune deposit formation in individuals with lupus, and that the predominant autoAb-autoAg interaction(s), in a given individual, influence both the morphologic and the clinical expression of nephritis. In the investigation of pathogenic autoAb (such as, V gene analysis), therefore, all autoAb are not equal. Consideration should include *both* the capacity of individual Ig to transfer disease *and* the morphologic and clinical expression of disease that results from this transfer. Furthermore, consideration of these principles should also be applied when planning specific therapy aimed at interrupting the immunologic pathways leading to the production of pathogenic autoAb in individuals with lupus.

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