

STAGES OF RENAL ONTOGENESIS IDENTIFIED BY MONOCLONAL ANTIBODIES REACTIVE WITH LYMPHOHEMOPOIETIC DIFFERENTIATION ANTIGENS*

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The antigenic complexity of fetal and mature human kidney has been the subject of recent investigation (1-5). Not unexpectedly, fetal kidney has been found to share some antigenic determinants with mature kidney (2-5) and to diverge with regard to others (1). These studies were facilitated by the characteristic pattern of metanephrogenesis whereby relatively mature nephrons are concentrically layered by less mature nephrons. Consequently, fetal and infant kidneys contain nephrons at all stages of development, the most primitive residing in a subcapsular location and the most mature residing in the juxtamedullary region.

The immunohistochemical probes used to investigate such tissues have largely consisted of heteroantisera and monoclonal antibodies developed after immunizations with renal tissue extracts (1-9). The likelihood that some antigens would remain undisclosed by this approach prompted our investigation using monoclonal antibodies produced by immunization of mice with extrarenal immunogens. Specifically, we have examined fetal and adult kidney tissues by indirect immunofluorescence using monoclonal antibodies directed against defined populations of lymphohemopoietic cells. These studies demonstrate a convergence between antigenic determinants of B lymphocytes and leukemic cells on the one hand and epithelial cells of fetal and adult kidney on the other, and suggest that acquisition or loss of these determinants can be related to the various stages of nephron differentiation and development.

Materials and Methods

Source of Tissue. Fetal kidney tissue was obtained from two abortuses each of 11-, 15-, 17-, and 21-wk gestations and from one abortus of 18 wk (estimated by crown-to-rump length). Mature human kidney tissue was obtained from normal portions of kidney removed from five patients with renal tumors or renal artery stenosis. All tissues were snap-frozen in precooled isopentane and stored at -70°C until use.

Monoclonal Antibodies. Mouse monoclonal antibodies derived from hybridoma cell lines were used to identify renal tissue antigens. Their subclass, specificity, and source are indicated in Table I. The following monoclonal immunoglobulins derived from mouse myeloma cell lines were used as controls: UPC10 (IgG_{2a}), MOPC 195 (IgG_{2b}), MOPC 104 E (IgM), and MOPC 21 (IgG₁) (Litton Bionetics, Kensington, MD).

Immunofluorescence Techniques. Kidney tissues were processed for immunofluorescence as

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TABLE I
Source Subclass and Specificity of Monoclonal Antibodies

Antibody	Mouse Ig subclass	Specificity	Reference
TA-1*	IgG _{2a}	Monocytes T lymphocytes	10
OKT3‡	IgG _{2a}	T lymphocytes	11
OKT4‡	IgG _{2b}	Helper/inducer T lymphocytes	12
OKT6‡	IgG ₁	Thymocytes	13
OKT8‡	IgG _{2a}	Suppressor/cytotoxic T lymphocytes	13
OKT10‡	IgG ₁	Thymocytes, activated T cells	14
OKIa1‡	IgG ₂	HLA-DR	15
B1§	IgG _{2a}	B cells	16
BA-1*	IgM	B cells, PMN	17
BA-2*	IgG ₃	Leukemia-associated antigen	18
BA-3*	IgG _{2b}	Leukemia-associated antigen	19
J5§	IgG _{2a}	Leukemia-associated antigen	20
MBM7*	IgG ₁	All renal basement membranes	4
MBM21*	IgG ₁	Proximal tubule basement membrane	4

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previously reported (21). 4- μ m sections were prepared on a Lipshaw cryostat (Lipshaw Manufacturing Co., Detroit, MI), air-dried, acetone-fixed for 10 min, and then washed with phosphate-buffered saline (PBS),¹ pH 7.4. Sections were reacted with the appropriate dilution of a monoclonal antibody, washed, and reacted with fluorescein isothiocyanate (FITC)-conjugated F(ab')₂ rabbit anti-mouse IgG (heavy and light chains), followed by FITC-F(ab')₂ goat anti-rabbit IgG (heavy and light chains) (N. L. Cappel Laboratories, Inc., Cochranville, PA). The double fluorochrome layer was used to achieve immunofluorescence enhancement, although specific fluorescence was discernible with a single fluorochrome layer.

In fetal kidney tissues, four stages of glomerular development were identified: (a) vesicle; (b) S body; (c) developing capillary loop stage; and (d) mature stage (22).

Comparison of Renal Antigenic Distributions. To compare the location of determinants recognized by BA-1 with those recognized by BA-2, BA-3, and J5, mixtures of BA-1 with each of the other antibodies were prepared at optimal end titer dilution. Renal tissue sections were reacted with one of these monoclonal antibody solutions, followed by a mixture of FITC F(ab')₂ goat anti-mouse IgM (μ chain) (which reacts with BA-1) and tetramethyl rhodamine isothiocyanate (TRIC)-conjugated goat anti-mouse IgG (Fc) (which identifies J5, BA-2, and BA-3) (N. L. Cappel Laboratories, Inc.) at optimal end titer dilutions. Control sections were reacted with the fluorochrome mixture alone without prior application of monoclonal antibodies.

Location of Antigenic Determinants at Serial Stages of Nephrogenesis. The relationship between antigenic expression and ontogenic stage was investigated by reacting fetal and adult kidney tissue sections with BA-1, BA-2, BA-3, or J5 and MBM7 (directed against glomerular basement membrane [GBM] and tubular basement membrane [TBM]) (4), followed by a double fluorochrome layer. Localization of antigen in proximal and distal tubules was determined morphologically as well as by staining sections with mixtures of the several anti-B and leukemic cell antibodies with MBM21 (which reacts only with proximal TBM). Additional sections were reacted with monoclonal antibody, then TRIC F(ab')₂ rabbit anti-mouse IgG (heavy and light chains) (N. L. Cappel Laboratories, Inc.), followed by FITC rabbit anti-human Tamm-Horsfall protein (ascending limb of the loop of Henle). The preparation and characterization of FITC anti-Tamm-Horsfall antibody has been previously described in detail (3).

¹ *Abbreviations used in this paper:* CALLA, common acute lymphoblastic leukemia antigen; FITC, fluorescein isothiocyanate; FITC *E. coli*, fluoresceinated *E. coli*; GBM, glomerular basement membrane; GVBS⁺⁺, veronal-buffered saline containing CaCl₂, MgCl₂, and gelatin; PBS, phosphate-buffered saline; TBM, tubular basement membrane; TRIC, tetramethyl rhodamine isothiocyanate.

Characterization and Relative Tissue Binding of J5 and BA-3 Antibodies The locations of J5 and BA-3 immunofluorescence were compared on parallel sections of fetal and adult kidney tissues. The differential tissue avidity of J5 and BA-3 was evaluated as follows. (a) Sections of adult kidney tissue were reacted with a mixture of J5 (IgG_{2a}) and BA-3 (IgG_{2b}) at optimal end titer dilutions, followed by rabbit anti-mouse IgG_{2a} or rabbit anti-mouse IgG_{2b} (Littion Bionetics) and then FITC F(ab')₂ goat anti-rabbit IgG. (b) Sections of adult kidney tissue were reacted sequentially in four layers with BA-3, J5, rabbit anti-mouse IgG_{2a}, or rabbit anti-mouse IgG_{2b} and FITC F(ab')₂ goat anti-rabbit IgG; or with J5, BA-3, rabbit anti-mouse IgG_{2a}, or rabbit anti-mouse IgG_{2b} and FITC F(ab')₂ goat anti-rabbit IgG. (c) To determine whether BA-3 could be eluted from kidney by J5, sections of kidney tissue previously reacted with BA-3 for 30 min (25°C) were washed with PBS, layered with 20 μ l of J5 at various dilutions, and incubated in a moist chamber at 25°C for 30 min. The droplet remaining on each section was then transferred to another acetone-fixed, unstained section of kidney tissue. After incubation for 30 min (25°C), the section receiving the transferred antibody solution was washed with PBS and reacted with rabbit anti-mouse IgG_{2b} (reactive with BA-3) or rabbit anti-mouse IgG_{2a} (reactive with J5), followed by FITC F(ab')₂ goat anti-rabbit IgG. (d) Sections were reacted with BA-3, washed, and then reacted with J5 as described above. Before transfer of the droplet, rabbit anti-mouse IgG_{2a} was added to precipitate any remaining J5 and then the droplet was transferred to another tissue section, which was stained for binding of BA-3 with rabbit anti-mouse IgG_{2b}, followed by FITC F(ab')₂ goat anti-rabbit IgG.

These studies were controlled and standardized by preparation of tissue sections reacted sequentially with (a) all antibody layers described above but omitting the first monoclonal antibody layer; (b) BA-3 or J5, rabbit anti-mouse IgG_{2a} or IgG_{2b}, and FITC F(ab')₂ goat anti-rabbit IgG; (c) BA-3 mixed with mouse myeloma IgG_{2a} (equal concentration to that of optimally diluted J5 in milligrams per milliliter), rabbit anti-mouse IgG_{2a}, or IgG_{2b} and FITC F(ab')₂ goat anti-rabbit IgG; (d) BA-3 and PBS or mouse myeloma IgG_{2a}, followed by droplet transfer and layering of anti-mouse and anti-rabbit sera as described above.

C3b Receptor Activity. The assay of C3b receptor activity on human kidney tissue sections using C3b-coated fluoresceinated *Escherichia coli* (FITC *E. coli*) has been previously described in detail (23). Briefly, heat-killed *E. coli* (O22:H16) suspended in 0.15 M NaCl at 5×10^9 organisms/ml were added to a solution of FITC (BBL Microbiology Systems, Cockeysville, MD). 0.113 mg/ml of 0.5 M carbonate/bicarbonate buffer (pH 9.0) at a ratio of 0.0045 mg FITC to 5×10^9 *E. coli*. The suspension was incubated for 16 h with gentle agitation and then washed four times with veronal-buffered saline (pH 7.5) containing CaCl₂ (0.15 mM), MgCl₂ (1.0 mM), and 0.1% gelatin (GVBS⁺⁺), and stored at -70°C until use. A suspension was then prepared that consisted of equal parts of FITC *E. coli* and fresh (*E. coli*-absorbed) human serum. The suspension was incubated and gently agitated at 37°C for 15 min, and washed with GVBS⁺⁺ (4°C), and the concentration of organisms was adjusted to 10^{10} /ml. Control suspensions were prepared as described above containing FITC *E. coli* in GVBS⁺⁺ without addition of absorbed human serum or with addition of heat-inactivated (56°C, 30 min) absorbed human serum.

This methodology was adapted to the present investigation by application to unfixed tissue sections of MBM7 or mixtures of MBM7 and BA-1, BA-2, BA-3, or OKIa1, followed by TRIC F(ab')₂ rabbit anti-mouse IgG, TRIC F(ab')₂ goat anti-rabbit IgG, and then by reaction of these sections with C3b-coated FITC *E. coli*. Control preparations included (a) reaction of unfixed tissue sections with C3b-coated FITC *E. coli* without prior application of antisera, and (b) reaction of fluorochrome-stained and unstained tissue sections with FITC *E. coli* suspensions prepared without addition of human serum or with addition of heat-inactivated (56°C, 30 min) *E. coli*-absorbed human serum.

Immunofluorescence Microscopy. To retard the fading of fluorescence, *p*-phenylenediamine in PBS-glycerol was applied to fluorochrome-stained sections (24). Separate studies using these and other antisera on human kidney tissue revealed no effect of this compound on antibody specificity (J. Platt, unpublished observation). Tissues were examined with a Zeiss Universal microscope (Carl Zeiss, Inc., New York) equipped for epifluorescence with appropriate filters and dichroic mirrors.

Standardization of Reagents and Immunofluorescence Controls. The appropriate dilutions of mono-

clonal antibodies were determined using the amplified immunofluorescence method described above on frozen sections of human lymphoid or renal tissue. Mouse myeloma antibodies were used at dilutions that approximated the optimal dilution of hybridoma-derived antibodies in milligrams of purified Ig per milliliter. Immunofluorescence controls included the following. (a) Each tissue was reacted with the various combinations of FITC-conjugated and TRIC-conjugated reagents without prior application of monoclonal antibodies. (b) Where mixtures of monoclonal or polyclonal antibodies were used, control sections were prepared on which these antibodies were layered sequentially. (c) Fc receptor binding was excluded by the application of mouse myeloma antibodies of the same Ig subclass as the hybridoma-derived antibodies used in the study, by the use of F(ab')₂ fluorochromes whenever possible, and by preparation of control sections on which the last fluorochrome layer applied consisted of F(ab')₂ antibody. The controls used in studies comparing J5 and BA-3 are described in detail above.

Results

Thymic and Mature T Cell Antigens. Fetal and adult kidney tissues were examined for the presence of thymic and mature T cell antigens using the following monoclonal antibodies: TA-1 (monocytes and T cells), OKT3 (mature thymocytes and T cells), OKT4 (common and mature thymocytes and helper/inducer T cells), OKT8 (common and mature thymocytes and suppressor/cytotoxic T cells), OKT6 (common thymocytes), and OKT10 (early, common, and mature thymocytes and activated T and B lymphocytes). No reactivity of glomerular, tubular, or vascular structures was noted. However, each monoclonal reagent reacted with occasional interstitial cells in fetal and adult tissues. In general, these cells in 11-, 15-, and 18-wk fetuses were large (similar in size to monocytes) and weakly fluorescent. Some mononuclear cells identified by OKT6 at 11 wk and OKT10 at 11 and 15 wk appeared to contain phase-dense cytoplasmic granules. Tissue sections of the 18-wk fetal kidney contained a few smaller mononuclear cells that were reactive with OKT4 and OKT10. In 21-wk fetal kidney tissue, each of the antibodies identified interstitial cells of small and moderate size that were greater in number and staining intensity than those observed in more primitive tissues.

BA-1. BA-1 is known to identify a cell surface antigen expressed by normal and malignant B cells at multiple stages of differentiation and also by granulocytes (17). In sections of fetal kidney, it reacted with all cells of the primitive subcortical nephron and ureteral bud (vesicle and S-body stages) (Fig. 1). Glomerular development was characterized by loss of antigen from the visceral glomerular epithelium and proximal tubule (Figs. 1 and 2). Thus, fetal tissues of all gestations displayed a gradient of immunofluorescence for BA-1 such that primitive subcortical nephrons appeared intensely and diffusely stained, whereas centripetal fields (and adult tissues) revealed relatively weak fluorescence restricted to Bowman's capsule epithelium and the distal nephron (Fig. 1). The antigen in the vesicle and S body seemed to be associated with the plasma membrane, whereas that of more mature tubules was located in the cytoplasm and brush border.

To determine more precisely which mature tubules were identified by BA-1 sections of adult kidney tissue were reacted with BA-1 and counter-stained with J5, BA-3 (proximal tubular epithelium), or MBM21 (basement membrane of proximal tubule and loop of Henle), followed by TRIC goat anti-mouse IgG (Fc), or with FITC rabbit anti-Tamm-Horsfall protein (epithelium of thick ascending limb and early distal tubule). These studies revealed that BA-1 was located in the distal part of the nephron by virtue of (a) lack of association with proximal tubules having reactivity for J5 and

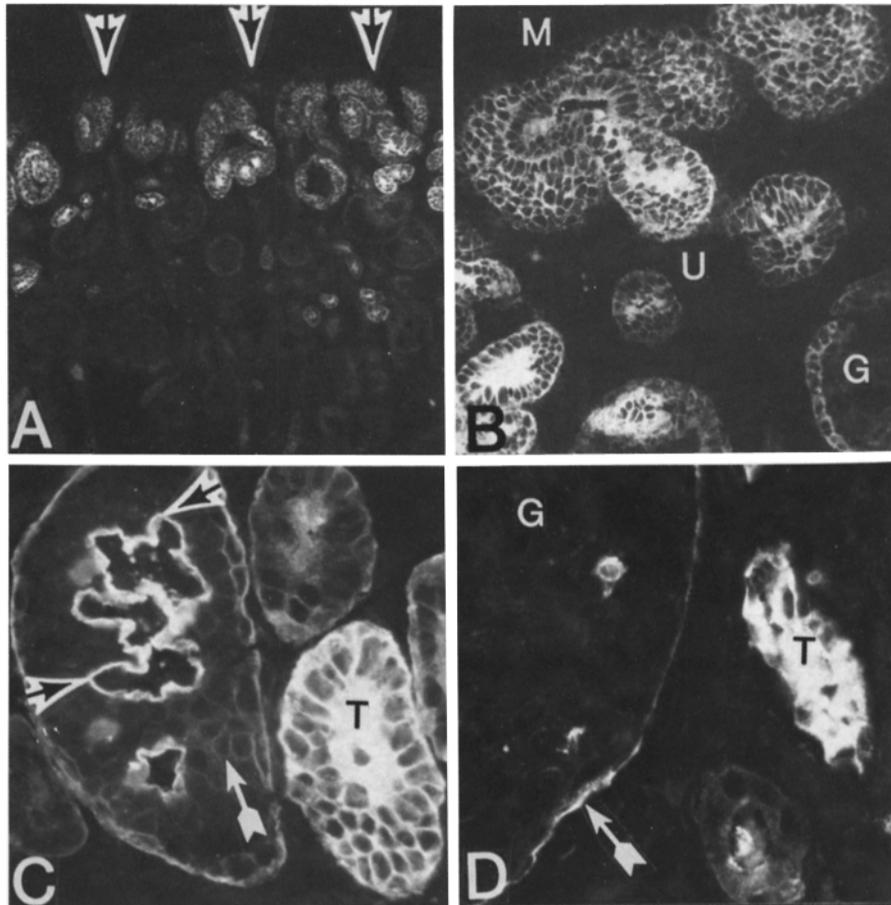


FIG. 1. BA-1 reactivity of fetal (A-C) and adult (D) human kidney tissue. (A) Primitive vesicles, S forms, and ureteral bud structures near renal capsule (arrowheads) identified by BA-1 in an 11-wk fetus. In more developed glomeruli in the deep cortex, reactivity is limited to Bowman's capsule and distal nephron ($\times 70$). (B) An early vesicle form in an 11-wk fetal tissue is reactive with BA-1 in cells of the ureteral bud (above U) and in the mesenchyme (below M) condensing about it. A more developed glomerulus (G) has reactive parietal epithelium ($\times 115$). (C) Early capillary loop stage in 21-wk fetal kidney stained with a mixture of BA-1 and a monoclonal antibody to human renal basement membrane (MBM7). MBM7 identifies Bowman's capsule and GBM (arrowheads). At this stage, parietal epithelium is reactive with BA-1, whereas visceral epithelium has lost or nearly lost (arrow) reactivity with this antibody. An adjacent distal tubule (T) is highly reactive with BA-1 ($\times 445$). (D) BA-1 fluorescence of mature human kidney tissue is limited to the parietal epithelium of Bowman's capsule (arrow) and a distal tubule (T). A granulocyte in the glomerulus (G) is also identified ($\times 445$).

BA-3; (b) infrequent expression on proximal cortical tubules against which MBM21 is directed; (c) association with some medullary tubules containing Tamm-Horsfall protein (although most tubules reacting with BA-1 were located in a more distal site).

Anti-B1. Anti-B1 antibodies recognize a population of B cells that is functionally and developmentally similar to that identified by BA-1 (16). Although interstitial cells reactive with anti-B1 were noted in kidney tissues as early as 15 wk gestation, parenchymal B1 expression was not observed in tissue less mature than 21 wk. In 21-

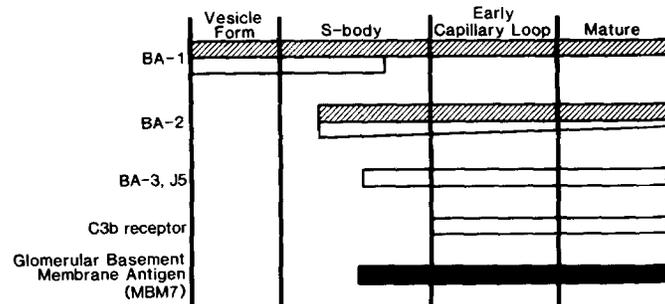


FIG. 2. Reactivity of parietal and presumptive parietal epithelium (hatched bars) and visceral and presumptive visceral epithelium (open bars) in developing human glomeruli with monoclonal antibodies BA-1, BA-2, and BA-3/J5 and with human C3b-coated *E. coli*. Four developmental stages of fetal glomeruli (vesicle form, S body, early capillary loop, and mature) are indicated as previously described (22). Acquisition of a human GBM antigen (reactive with monoclonal antibody MBM7) is shown for reference (solid bar).

wk tissues, granular B1 fluorescence was noted in the cytoplasm of ureteral bud and collecting duct epithelium. Parenchymal reactivity was not observed in normal adult human kidney.

BA-2. BA-2 antibody is known to react with most acute lymphoblastic leukemia cells (18), as well as with a large number of human tumors of epithelial and neural crest origin (T. LeBien, unpublished observations). In fetal tissue, ureteral bud structures revealed intense fluorescence. Glomerular and tubular reactivity were first noted in the S body after capillary invasion. The vesicle and primitive S form were unreactive (Fig. 3). Development of the nephron was associated with gradual but incomplete disappearance of reactivity from the visceral glomerular epithelium and proximal tubule and with persistence in epithelial cells of Bowman's capsule and distal tubule (Figs. 2 and 3). Immunofluorescence in adult kidney tissue was intense along the inner aspect of Bowman's capsule and tubular segments distal to the distal convoluted tubule, where individual tubular cells or internal and apical cell membranes were notably identified. A moderate number of interstitial mononuclear cells displayed plasma membrane fluorescence. Fetal as well as adult arterial smooth muscle and capillaries also reacted with BA-2.

J5 and BA-3. J5 and BA-3 antibodies are both directed against the common acute lymphoblastic leukemia antigen (CALLA) (19, 20). The immunofluorescence pattern of each antibody on renal tissue sections was indistinguishable from that of the other. The most primitive of glomeruli at vesicle and S-body stages were consistently unreactive with BA-3 and J5 (Fig. 4). In contrast, the antibodies distinctly stained the visceral epithelium of more mature fetal and adult glomeruli (Figs. 4 and 5). Additionally, BA-3 and J5 reacted with the brush border of the proximal tubules associated with the more mature nephrons. The glomerular endothelium, basement membrane, parietal epithelium, and distal tubule of fetal and adult kidney tissue were never reactive with BA-3 or J5.

To determine the relationship between glomerular development and epithelial expression of CALLA, sections of fetal kidney tissue were reacted with BA-3 or J5 and counter-stained with MBM7 (directed against GBM, TBM, and Bowman's capsule). These studies revealed that BA-3 and J5 reactivity was confined to glomerular epithelial cells closely associated with basement membrane and confirmed the absence

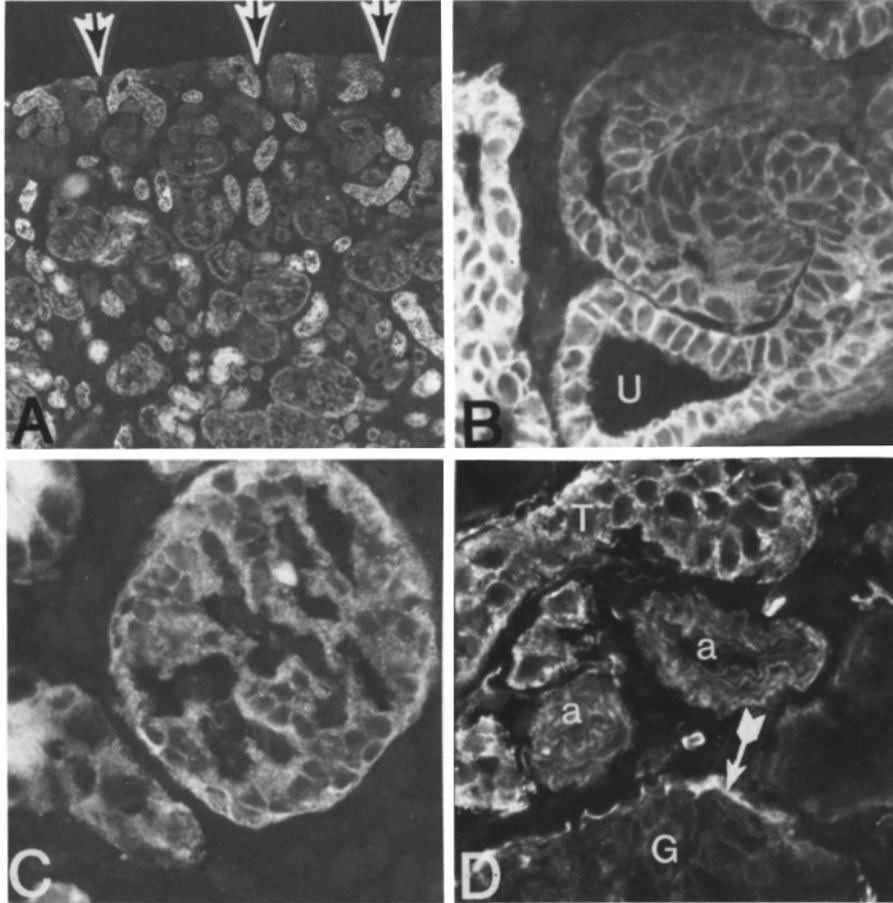


FIG. 3. Reactivity of fetal (A-C) and mature (D) kidney tissues with BA-2. (A) 21-wk fetal kidney tissue in which primitive ureteral bud elements at outer cortex (arrowheads) are intensely reactive with BA-2. In more mature nephrons in the deep cortex, Bowman's capsule and distal tubules are most notably reactive ($\times 70$). (B) S form in 21-wk fetal kidney tissue is identified by BA-2. The associated ureteral bud below (U) and to the left is also reactive ($\times 445$). (C) Capillary loop-stage glomerulus in 18-wk fetal kidney has BA-2 fluorescence along parietal epithelium. Fluorescence of visceral epithelium is beginning to wane. Adjacent distal tubules are also reactive for BA-2 ($\times 445$). (D) Adult kidney tissue reacted with BA-2 reveals fluorescence of parietal epithelium of Bowman's capsule (arrow) and weak reactivity of the visceral epithelium in the glomerulus (G). Two arterioles (a) contain smooth muscle reactive with BA-2. The epithelium of a distal tubule (T) is also reactive ($\times 445$).

of epithelial staining before basement membrane formation (Fig. 2). Furthermore, CALLA fluorescence was most intense at the lateral and apical aspects of epithelium in direct contact with basement membrane. No glomeruli that evidenced CALLA lacked identifiable GBM; however, in some primitive glomeruli, CALLA was identified on the more centrally located visceral epithelium and not on the peripheral visceral epithelium (Fig. 4).

Sections of adult and fetal kidney stained with BA-3 or J5 were counter-stained with MBM21, BA-1, and rabbit anti-human Tamm-Horsfall protein. All tubules reactive for CALLA were also reactive for MBM21. The point at which CALLA

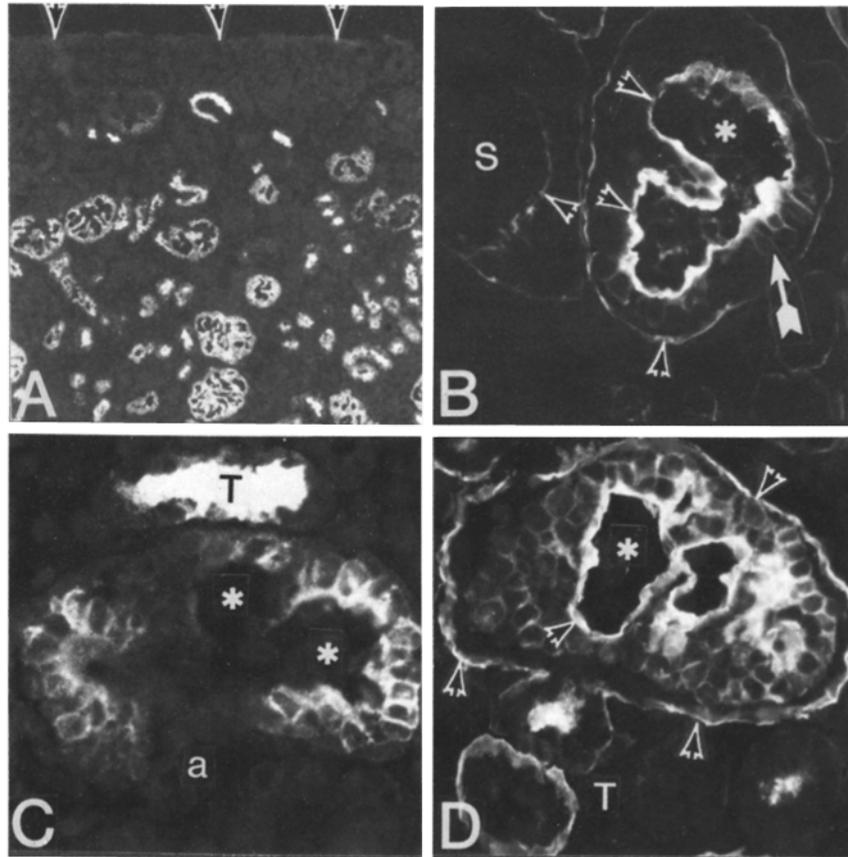


FIG. 4. BA-3 reactivity with 21-wk kidney fetal tissue. (A) Relatively mature nephrons in the inner cortex are reactive with BA-3, whereas primitive nephrons adjacent to the renal capsule (arrowheads) are not ($\times 70$). (B) Fetal kidney tissue reacted with MBM7 directed against renal basement membranes (arrowheads denote GBM and Bowman's capsule) and also with BA-3. An early capillary loop stage glomerulus (right) contains some visceral epithelium, which is developing BA-3 reactivity (arrow), whereas epithelium associated with the upper capillary loop (asterisk denotes capillary lumen) is unreactive. An adjacent S form (S) does not react with BA-3 ($\times 445$). (C) A more mature capillary loop-stage glomerulus containing BA-3 reactive visceral epithelium in proximity to capillary loops (asterisks denote capillary lumina); the afferent/efferent arteriole (a) is not reactive, whereas the brush border of a proximal tubule (T) demonstrates striking fluorescence ($\times 445$). (D) A maturing capillary loop-stage glomerulus whose basement membranes (GBM, Bowman's capsule) are identified by MBM7 (arrowheads). Abundant visceral epithelium surrounding capillary loops (asterisks) is reactive with BA-3, whereas the parietal epithelium is not. Below the glomerulus are two tubules (T): a proximal tubule, whose brush border is identified by BA-3 and the underlying basement membrane by MBM7; immediately beneath the proximal tubule is a distal tubule whose basement membrane is reactive with MBM7 ($\times 445$).

antigen ceased to be present was observed on several fortuitous longitudinal sections of descending tubules. Most medullary tubules were reactive with MBM21 but not with J5 or BA-3. Tubules with CALLA fluorescence were not reactive with BA-1 or anti-Tamm Horsfall protein antibodies.

Prior investigations have suggested that J5 and BA-3 are directed against the same epitope of the common acute lymphoblastic leukemia antigen, J5 having the greater

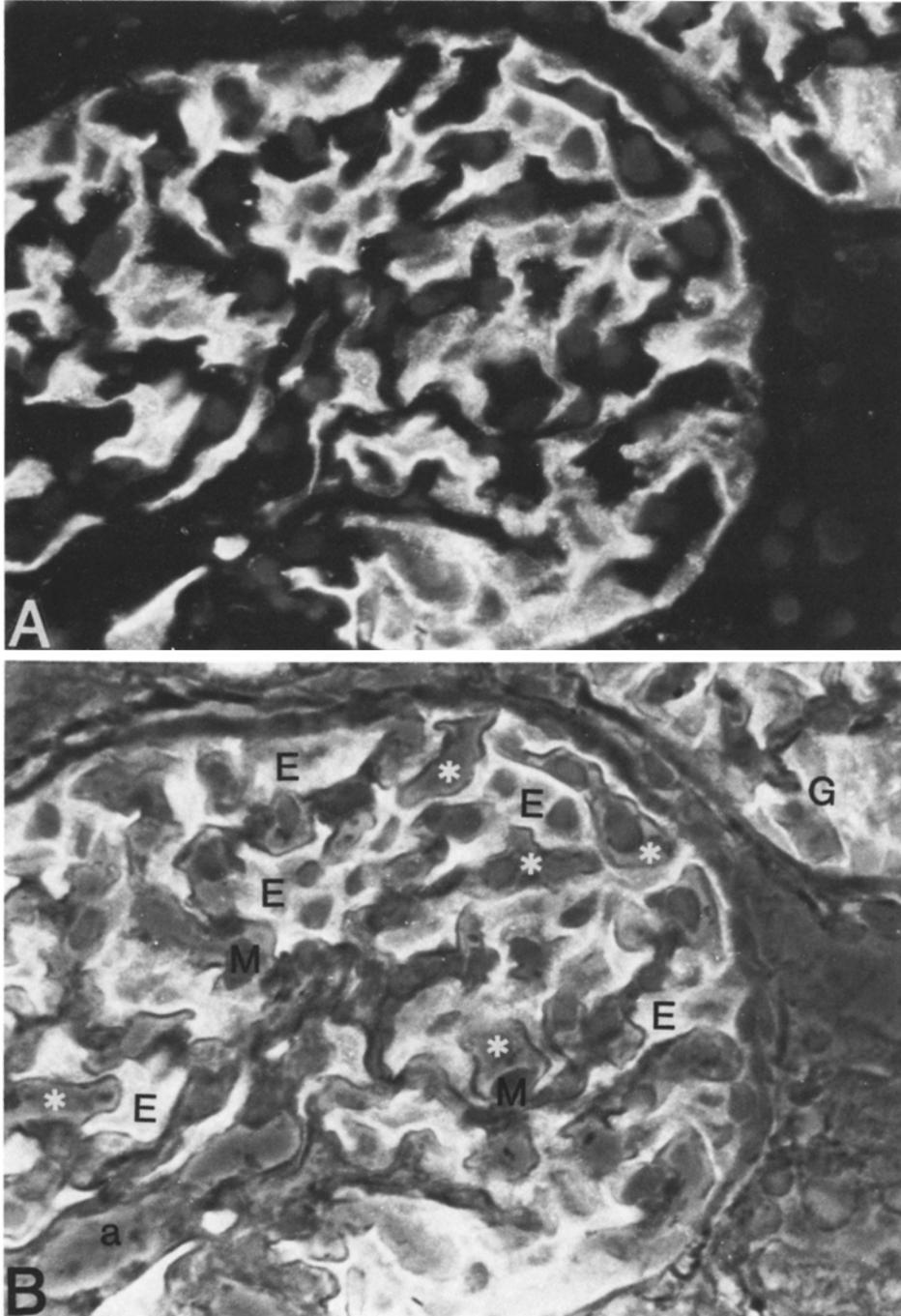


FIG. 5. Reactivity of mature glomerulus with J5. (A) Immunofluorescence photomicrograph of mature glomerulus reacted with J5. (B) Immunofluorescence photomicrograph of the same glomerulus with phase-contrast enhancement to identify capillary lumina (asterisks) surrounded by phase-dense glomerular basement membrane and J5-reactive visceral epithelium (E). The mesangia (M) and arteriolar pole (a) are unreactive. An adjacent glomerulus (G) reveals similar features ($\times 665$).

affinity (19). Our findings that support this hypothesis include the following: (a) the distribution of renal determinants recognized by the two antibodies is the same; (b) kidney tissue sections reacted with a mixture of optimally diluted J5 and BA-3 antibodies reveal intense J5 and minimal BA-3 fluorescence; (c) the application of J5 to tissue sections previously reacted with BA-3 abolishes BA-3 reactivity, whereas BA-3 application to sections previously reacted with J5 does not affect the intensity of J5 fluorescence; (d) that BA-3 is eluted from tissue rather than masked by application of J5 was conclusively shown by droplet transfer experiments which consistently revealed the presence of BA-3 antibodies in the eluted droplet and enhancement of BA-3 reactivity by immunoabsorption of J5.

HLA-DR. HLA-DR antigen was detected in adult kidney tissue on peritubular capillaries and glomerular endothelium and mesangium as previously reported (25–27). The expression of this antigen in fetal kidney tissues varied with the gestational age of the fetus. In early gestation (11 wk), weak focal fluorescence of peritubular capillaries and focal segmental fluorescence of glomerular capillaries was observed. At 15 wk, mesangial reactivity was first noted. With advancing gestational age, a greater proportion of glomerular and peritubular capillaries reacted with OKIa1 and the fluorescence appeared progressively more intense (Fig. 6). The expression of HLA-DR antigen did not correlate with the stage of nephron development. The vesicles and S bodies in more mature fetuses (21 wk) were surrounded by Ia-reactive capillaries and the earliest evidence of capillary in-growth was witnessed as distinct capillary reactivity at the proximal crevice of the S body, whereas the vesicles and S bodies in less mature fetuses revealed only weak focal fluorescence in these locations. Some round

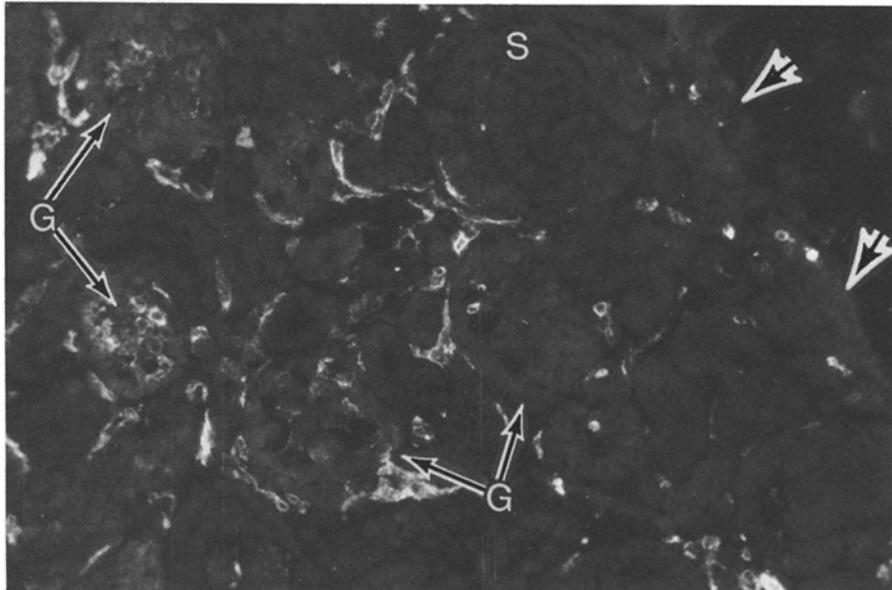


FIG. 6. Fetal kidney tissue (21-wk) reacted with a monoclonal antibody (OKIa1) that recognizes human Ia-like antigen. Outer cortex (arrowheads) contains primitive glomeruli and one distinct S form (S) surrounded by capillaries and interstitial cells, some of which are weakly fluorescent. Centrifugally located more mature glomeruli (G) contain capillaries, a variable proportion of which react with OKIa1 ($\times 560$).

cells at the center of primitive glomeruli displayed Ia reactivity along the plasma membrane, as did large round interstitial cells that appeared to be associated with peritubular capillaries. Sections of fetal kidney to which a mixture of BA-3 and OKIal was applied revealed some primitive glomeruli in which capillary staining for Ia was observed without evidence of CALLA expression by the contiguous visceral epithelium.

C3b Receptor. The relationships between gestational age, metanephric development, and glomerular C3b receptor activity were examined in fetal kidney tissues. Unfixed tissue sections were reacted sequentially with MBM7 (basement membrane marker) or OKIal or mixtures of MBM7 and BA-1, BA-2, or J5, followed by a double fluorochrome layer (TRIC) and finally with C3b-coated FITC *E. coli*.

C3b receptor activity, indicated by glomerular aggregation of FITC *E. coli*, was observed in all tissues examined (11 wk gestation to adult). In fetal tissues, aggregation was only noted in glomeruli with well-developed basement membranes (as indicated by MBM7 reactivity) and was not present at the vesicle and S-body stages (Fig. 2).

In fetal tissues stained with BA-1/MBM7 or BA-2/MBM7 mixtures and FITC *E. coli*, C3b receptors were not observed in the subcortical vesicles and S forms intensely reactive with BA-1 and BA-2. In contrast, fetal kidney reacted with a J5/MBM7 mixture revealed that all glomeruli having FITC *E. coli* aggregates were reactive with J5. However, the converse was not observed in that some less mature glomeruli containing visceral epithelium weakly reactive with J5 did not evidence C3b receptors.

In tissue sections prepared with OKIal and FITC *E. coli*, aggregates were noted only in glomeruli having substantial OKIal fluorescence. Primitive glomeruli containing little OKIal reactivity did not appear to have C3b receptors.

Control studies failed to reveal any inhibition of C3b receptor activity by prior binding of monoclonal antibodies.

Discussion

The studies reported here demonstrate that a series of monoclonal antibodies that recognize cell surface molecules expressed on B cells and leukemic cells also identify well-defined stages of metanephric development. Furthermore, the pattern of metanephric cell differentiation can be related to the acquisition of a GBM antigen and a C3b receptor activity, but less clearly to the expression of Ia-like antigen.

Human metanephric development begins during the fifth week of gestation. It is characterized by aggregation of nephrogenic mesenchyme opposite branches of the ureteral bud (vesicle stage), formation of the primitive S body consisting of presumptive glomerular and tubular epithelium, followed sequentially by capillary ingrowth, GBM formation, and differentiation of glomerular parietal, visceral, and tubular epithelium. Development proceeds in a centrifugal fashion as newly forming glomeruli are layered in apposition to more mature glomeruli. Thus, from the eighth gestational week, fetal kidney tissue is found to contain nephrons at all stages of development.

Most prior immunohistologic studies (1-3, 6-9) of the developing kidney have used heteroantisera derived from immunization with renal tissue extracts to demonstrate the presence of antigens expressed at various developmental stages by the glomerular epithelium, basement membrane, and tubular epithelium. These studies have demonstrated some kidney-specific antigens. However, the number of recognizable anti-

gens was necessarily limited to immunogenic determinants not expressed on the various extrarenal tissue extracts used for immunoabsorption (1).

Affinity-purified heteroantisera directed against well-characterized antigens extracted from nonrenal tissues have been very useful in locating extracellular renal differentiation antigens such as type IV collagen, fibronectin, and laminin (28-30). However, this approach requires prior identification and purification of the antigen. The use of monoclonal antibodies to identify renal tissue antigens (4) obviates the removal of potentially useful antibodies by immunoabsorption, assures specificity of the antibody probe, and permits use of antibodies against cellular antigens that are more readily characterized functionally than biochemically. However, these investigations have used antibodies produced by immunizations with renal tissue antigens and thus were limited by the immunogenicity of the extracts.

In this study we have used a number of monoclonal antibodies derived from immunizations of mice with human lymphohemopoietic cells to investigate the extent to which antigens expressed by well-characterized populations of lymphoid cells are concomitantly expressed in fetal and mature kidney tissue. One previous report (31) and our coincidental observations (21) suggested that this approach could be revealing.

Reaction of fetal and adult kidney tissues with monoclonal antibodies that recognize T cells (OKT3, TA-1), T cell subsets (OKT4, OKT8), and thymocytes (OKT6, OKT10) did not reveal renal parenchymal fluorescence. These antibodies did identify occasional interstitial cells, which were generally larger, more numerous, and less intensely fluorescent in fetal than in adult tissues. These cells may be precursors of mature lymphoid cells, which sparsely populate normal mature interstitium (21). However, their identity as such cannot be proven without investigation of the functional correlates of their plasma membrane antigens. Still, it is of interest that renal tissues from fetuses of 11-18-wk gestations contained notably more interstitial cells that reacted with antibodies directed against thymocytes (OKT6, OKT10) than did more mature tissues.

The application of antibodies that recognize leukemia-associated antigens (BA-2, BA-3, and J5) and B cell antigens (BA-1 and anti-B1) resulted in striking immunofluorescence of fetal and adult kidney tissues. BA-1 reacted with all immature renal epithelia and the ureteral bud. Nephron development was characterized by abrupt loss of BA-1 from all but glomerular parietal epithelium and distal tubular epithelium. Loss of BA-1 fluorescence was associated with the appearance of immunoreactive GBM and acquisition by visceral epithelium of CALLA. BA-2 reacted with all ureteral bud epithelium and with glomerular and tubular epithelia after capillary invasion of the S body. BA-2 fluorescence of visceral glomerular and proximal tubular epithelia gradually diminished with nephron maturation. These patterns of BA-1 and BA-2 reactivity were observed in fetal tissues of all gestations and thus reflected nephron differentiation rather than duration of gestation (Fig. 2).

Anti-B1 antibody reacted with the ureteral bud and some distal tubules of 21-wk fetal kidney tissues. Parenchymal fluorescence was not observed in kidneys from less mature fetuses or in the five mature human kidney tissues to which it was applied. Distal tubular reactivity was noted, however, in one diseased adult tissue (J. Platt, unpublished observation). Occasional interstitial cells were identified by B1 at gesta-

tions of ≥ 15 wk. Thus, the occurrence of B1 antigen appears developmentally related but gestationally limited.

The patterns of immunofluorescence observed with J5 and BA-3 antibodies were indistinguishable. Both antibodies reacted with the visceral epithelium of the glomerulus and the brush border of the proximal tubule. These data are consistent with a previous report of J5 reactivity in renal tissue (31). Expression of the antigen recognized by J5 and BA-3 was distinctly related to glomerular differentiation rather than gestation because antigenic distribution was the same in all fetal tissues (Fig. 2). Fluorescence was never observed at the vesicle and early S-body stages before GBM formation, but appeared first on glomerular visceral epithelial surfaces in proximity to the developing basement membrane recognized by a monoclonal antibody, MBM7. In more mature glomeruli, J5 and BA-3 identified all visceral epithelium. The presence of J5/BA-3-positive and -negative epithelium contacting the basement membrane in the same glomeruli suggests that CALLA expression occurs shortly after the GBM is formed. The glomerular polyanion, an epithelial cell surface sialoprotein, is also recognized early in the S-body stage of developing glomeruli (29, 32).

There are data that indicate that BA-3 and J5 are directed against the same epitope of the common acute lymphoblastic leukemia antigen, J5 having the greater affinity (19). Out studies of comparative tissue binding lend strong support to this contention.

The expression of HLA-DR in fetal and adult tissues was examined using OKIa1 antibodies that recognize a monomorphic determinant on human Ia-like, cell-surface glycoproteins. Prior investigation of Ia-like antigen distribution in the mature human kidney have used heteroantisera (25) and monoclonal antibody probes (26, 27). Our studies of adult kidney tissue yielded results similar to these reports: HLA-DR was detected on the glomerular capillary loops, endothelium of intraglomerular arterioles, mesangium, and peritubular capillaries.

OKIa1 reactivity was observed in all fetal tissues examined, the extent and fluorescence intensity increasing progressively with gestational age. Reactivity was sparsely distributed in 11-wk tissues occurring in focal segmental glomerular capillary loops and along some peritubular capillaries. The antibody also identified round cells associated with capillaries in the glomeruli and interstitium. These may be developing endothelial cells or lymphohemopoietic cells. Mesangial fluorescence was first noted in 15-wk fetal tissues. In 21-wk tissues, reactivity was noted in glomerular endothelia, mesangia, and peritubular capillaries. Thus, HLA-DR expression appeared to be related more to gestation than developmental stage.

The presence of receptors for C3b on the visceral epithelium of the glomerulus has been investigated using C3b-coated erythrocytes (33-36), bacteria (23, 37, 38), immune complexes (39), and monoclonal antibodies (40, 41), and has been reported to occur in human fetal kidney as early as the 12th gestational week. We investigated the association of C3b receptor activity with the antigenic characteristics of fetal glomeruli using C3b-coated, fluorescein-labeled *E. coli* applied to tissue sections that had been reacted with BA-1, BA-2, OKIa1, and J5. These studies indicated that development of C3b receptors is related to nephron differentiation and not gestation because mature glomeruli in all tissues examined evidenced C3b receptors, whereas the most immature glomeruli did not. Additionally, the expression of C3b receptors was associated with loss of BA-1 and BA-2 reactivity and acquisition of CALLA by visceral epithelium as follows. (a) Early-developing capillary loop-stage glomeruli with

C3b *E. coli* aggregates had little visceral epithelial reactivity for BA-1 and BA-2, whereas more primitive glomeruli strongly reactive with these antibodies never evidenced C3b receptors. (b) All glomeruli with C3b *E. coli* aggregates reacted with J5 and BA-3, whereas some immature glomeruli having segmental J5 fluorescence had no aggregation of C3b *E. coli*. It would thus appear that the epithelium of developing glomeruli sequentially acquire CALLA, lose BA-1 (and some BA-2) reactivity, and then acquire C3b receptors. That BA-1 and BA-2 antibodies did not block C3b receptor activity was indicated by the observation that glomeruli with C3b *E. coli* aggregates were similarly located on parallel sections with and without prior application of BA-1 and BA-2.

It is reasonable to ask whether any of the antigens we have described in fetal and adult tissues are filtered at the glomerulus and adsorbed to epithelial cell surfaces. This explanation is not likely to account for early fetal expression of the antigens recognized by BA-1, BA-2, and anti-B1 because these antigens occur most dramatically on the distal nephron before capillary invasion of the S body and connection of the ureteral bud to the primordial distal nephron. CALLA could be filtered by mature glomeruli and adsorbed to visceral epithelium. However, the segmental fluorescence of developing glomeruli and the known sieving properties of mature glomeruli (42, 43) suggest that the antigen is not simply filtered and adsorbed. On the other hand, we cannot exclude the possibility that tubular reactivity with these antibodies in mature kidneys results from adsorption of antigens shed by the glomerulus (44).

In view of prior observations that glomeruli contain C3b and perhaps Fc receptors (33-41), could our immunofluorescence findings reflect nonspecific or Fc receptor binding of the mouse monoclonal antibodies? We have examined all tissues with mouse myeloma antibodies of the same immunoglobulin subclasses as the hybridoma antibodies used and have not seen tissue reactivity. The absence of parenchymal staining with anti-T cell antibodies and the use of F(ab') fluorochromes serve as additional controls. Finally, the clearly distinguishable pattern of fluorescence obtained with each monoclonal antibody argues further against nonspecific reactivity.

Assuming the immunofluorescence findings we have described reflect a specific antibody-antigen reaction, there remains an important question: are the monoclonal antibodies reacting with identical macromolecules or fragments of macromolecules commonly expressed by lymphohemopoietic cells and renal parenchyma, or are our immunofluorescence findings a consequence of shared epitopes of dissimilar macromolecules (45)? Others (25) have isolated and characterized Ia-like antigen from human kidney tissues. Furthermore, a J5-reactive molecule with a molecular mass of ~90,000 daltons has been isolated from human kidney (31). We can provide no direct evidence that the antigens identified by BA-1 and BA-2 in fetal kidney are identical to those located on lymphohemopoietic cell surfaces. However, preliminary experiments with BA-2 indicate that a molecule of ~24,000 daltons, similar to that of p24 precipitated from leukemic cells with BA-2 (18), can be detected in human urine (T. LeBien and J. Platt, unpublished observations).

Our investigations have suggested that an antigenic convergence exists between B cells and leukemic cells on the one hand and parenchymal structures in fetal and adult kidneys on the other. Such convergence is not evident for the six anti-T cell antibodies studied. This observation is of interest because stem cells common to

myeloid and B lymphocytic lines have been recognized (46), whereas a stem cell common to B and T lymphocytes has not been identified.

It is intriguing that glomerular epithelium and lymphohemopoietic cells share at least four differentiation antigens, have plasma membrane C3b and Fc receptors, and reside in a microenvironment enriched for endothelial HLA-DR antigen and accessory cell function (47). Because studies in lower vertebrates have suggested that lymphohemopoietic stem cells may arise in proximity to nephrogenic mesoderm (48-52), there is reason to inquire whether lymphoid cells or their progenitors share unrecognized physiologic functions or ontogenic experiences with renal cells.

Summary

Differentiation antigens of T and B lymphocytes were sought in human fetal and adult kidney tissues with monoclonal antibodies by indirect immunofluorescence.

Antibodies that identify B cells (BA-1 and anti-B1) and leukemia-associated antigens (BA-2, BA-3, and J5) reacted with renal glomerular and tubular epithelium at characteristic stages of nephron development. BA-1 and BA-2 identified primitive epithelium of the glomerulus, and ureteral bud and nephron development was characterized by loss of BA-1 and BA-2 binding by visceral glomerular and proximal tubular epithelium. In contrast, J5 and BA-3 did not react with primitive epithelium but identified visceral and proximal tubular epithelium after appearance of the glomerular basement membrane and throughout subsequent nephron differentiation. Anti-B1 reacted with ureteral bud and distal nephron epithelium in more mature fetal tissues.

Monoclonal antibodies that identify populations of T cells and thymocytes did not react with parenchymal cells of fetal or adult kidneys. They did identify interstitial mononuclear cells whose size and relative numbers appeared gestationally related.

Monoclonal antibodies that recognize a human monomorphic HLA-DR determinant reacted with glomerular and peritubular capillaries as early as 11 wk of gestation. The distribution and density of HLA-DR expression appeared more related to gestation than nephron development.

The relationship between renal parenchymal expression of lymphohemopoietic antigens and glomerular acquisition of C3b receptor activity was determined using C3b-coated fluoresceinated *Escherichia coli*. In fetal tissues, C3b receptor activity appeared developmentally related to the loss of determinants recognized by BA-1 and BA-2 and to the appearance of J5 and BA-3 reactivity with visceral glomerular epithelium.

Tissue binding and comparative avidity of J5 and BA-3 antibodies was studied in a series of experiments, the results of which suggest that these antibodies are directed against the same epitope or closely related epitopes of the common acute lymphoblastic leukemia antigen.

The common expression of differentiation antigens and C3b receptors by cells of lymphohemopoietic lineage and renal epithelia suggests the possibility of heretofore unrecognized commonality of function or developmental experience.

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