Altered Hepatic Transport of Immunoglobulin A in Mice Lacking the J Chain

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Summary

We have created J chain knockout mice to define the physiologic role of the J chain in immunoglobulin synthesis and transport. The J chain is covalently associated with pentameric immunoglobulin (Ig) M and dimeric IgA and is also expressed in most IgG-secreting cells. J chain–deficient mice have normal serum IgM and IgG levels but markedly elevated serum IgA. Although polymeric IgA was present in the mutant mice, a larger proportion of their serum IgA was monomeric than was found in wild-type mouse serum. Bile and fecal IgA levels were decreased in J chain–deficient mice compared with wild-type mice, suggesting inefficient transport of J chain–deficient IgA by hepatic polymeric immunoglobulin receptors (pIgR). The pIgR-mediated transport of serum-derived IgA from wild-type and mutant mice was assessed in Madin-Darby canine kidney (MDCK) cells transfected with the pIgR. These studies revealed selective transport by pIgR-expressing MDCK cells of wild-type IgA but not J chain–deficient IgA. We conclude that although the J chain is not required for IgA dimerization, it does affect the efficiency of polymerization or have a role in maintaining IgA dimer stability. Furthermore, the J chain is essential for efficient hepatic pIgR transport of IgA.

The J chain is a 15-kD glycoprotein covalently linked by disulfide bonds with polymeric IgA (pIgA) and IgM, implicating this glycoprotein in the polymerization process (1). The role of the J chain in polymer formation and stabilization, however, remains uncertain (2). The J chain is also expressed in many IgG-secreting cells, although its function in these cells is unknown (1, 3, 4). Some studies have suggested that the J chain plays a role in polymeric Ig receptor (pIgR)–mediated transport of IgA (5–7), while other studies have questioned the J chain's significance in this process (8, 9). The hepatic pIgR transports pIgA out of the serum into the bile (10–13). In mice and rats, which express pIgR on their hepatocytes, hepatic transport of IgA into bile plays an important role in serum IgA clearance (10–15). Knowledge of the mechanism and regulation of secretory IgA transport is important to our understanding of host defenses against mucosally acquired pathogens and of the liver's role in clearing IgA–antigen complexes from the circulation.

Knockout mice lacking the J chain provide an excellent tool for defining the role of this polypeptide in Ig synthesis and transport. Most of the previous work investigating the function of the J chain in these processes has been done with purified preparations of Ig and J chain or with isolated cell lines. Using J chain–deficient mice created using gene targeting techniques, we demonstrate here that IgA dimerization can occur in vivo in the absence of the J chain. However, the J chain is required for the efficient transport of serum-derived IgA into bile.

All animal care was in accordance with insititutional guidelines. For invasive procedures, mice were anesthetized with Avertin (0.017 ml/g) i.p. before the procedure.

[1] *Abbreviations used in this paper:* DTT, dithiothreitol; ES, embryonic stem; FIAU, fialuridine; Jch, J chain mutation; MDCK, Madin-Darby canine kidney; neo^r, neomycin resistance gene; pIgA, polymeric IgA; pIgR, polymeric Ig receptor; SC, secretory component.

Materials and Methods

Preparation of Targeting Construct. The J chain gene isolated from a WEHI231.R lymphoma library in Charon 4A was kindly provided by Dr. M. Koshland (Department of Molecular and Cell Biology, University of California, Berkeley, CA). The neomycin resistance gene (neo^r) under the control of the phosphoglycerate kinase promoter was cloned into an Ava I site of a PstI–NcoI 9.3-kb genomic fragment containing the entire murine J chain gene subcloned in Bluescript SK II$^+$ (Stratagene Inc., La Jolla, CA). The phosphoglycerate kinase–*Herpes simplex* thymidine kinase gene was cloned into a SalI site in the polylinker.

ES Cell Transfection and Culture. The male embryonic stem (ES) cell line C1, (derived from mouse strain 129/SvJ by D. A. Connor), was cultured on irradiated mouse embryonic fibroblasts in supplemented DME with 500 U/ml LIF (Gibco Laboratories, Grand Island, NY). Construct DNA (16 μg) was transfected by electroporation (125 μF, 450 V; Gene Pulser; Bio-Rad Laboratories, Hercules, CA) into 2 × 10^7 C1 ES cells. Cells surviving G418 (230 μg/ml, Gibco Laboratories) and fialuridine (FIAU) (0.2 μM; Bristol Meyers Squib, Wallingford, CT), added 1 and 2 d after electroporation, respectively, were screened by Southern blot analysis as described previously (16) with a BamHI-SstI fragment shown in Fig. 1 *A*.

Northern Blot Analyses. RNA harvested from splenocytes stimulated with LPS (50 μg/ml; L-7261 Sigma Chemical Co., St. Louis, MO) and IL-5 (25 U/ml; Genzyme Corp., Cambridge, MA) was fractionated on a formaldehyde–agarose (1%) gel, transferred to GeneScreen (NEN Research Products, Boston, MA), hybridized with ^{32}P-labeled 1.1-kb BamHI–EcoRI J chain cDNA (a kind gift of Dr. M. Koshland) and murine actin probes, and washed as described previously (16).

Rabbit Anti–Human J Chain Antiserum. Recombinant J chain was synthesized and purified in *Escherichia coli* strain M15 (Qiagen, Chatsworth, CA) carrying an expression plasmid expressing the human J chain cDNA. Total RNA was prepared from human Dakiki cells (TIB 206; American Type Culture Collection, Rockville, MD) as described (17). Human J chain cDNA encoding the mature protein (i.e., exons 2–4, omitting the signal peptide) was obtained by reverse transcription-PCR using primer 5′-GCC AAG CTT TTA GTC AGG ATA GC-3′ for reverse transcription and subsequent PCR amplification, together with primer 5′-GGA GAT CTG AAG ATG AAA GG-3′. Primers were designed based on the published genomic sequence (18). Underlined sequences represent additional nucleotides corresponding to restriction sites HindIII and BglII that were used for subsequent cloning into the pQE9 (Qiagen) bacterial expression vector. Plasmid bearing *E. coli* were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma Chemical Co.). 3 h after induction, the protein was recovered from inclusion bodies, solubilized in 7 M urea, and purified by affinity chromatography on Ni2$^+$–chelate agarose beads. A 3-mo-old female New Zealand rabbit was primed with 200 μg of recombinant J chain in urea by subcutaneous injection and subsequently boosted monthly with the same amount of J chain.

Immunoglobulin ELISAs. IgA and IgG ELISAs were performed as described previously (19). For the IgM ELISAs, diluted samples were added to immunoplates (MaxiSorp; Nunc, Roskilde, Denmark) coated with 5 μg/ml goat anti–mouse IgM (Cappel Research Products, Durham, NC) in bicarbonate buffer and blocked with 3% BSA. IgM was detected with rabbit anti–mouse IgM alkaline phosphatase conjugate (Zymed Laboratories, Inc., South San Francisco, CA)/1 mg/ml *p*-nitrophenyl phosphate (Sigma Chemical Co.). Absorbance readings were compared with standard curves generated with IgM (PharMingen, San Diego, CA).

Protein Electrophoresis and Immunostaining for IgA. For native gels, samples were diluted in Tris/HCl buffer (pH 8.9) and electrophoresed on 10% polyacrylamide gels (CleanGel; Pharmacia Biotech, Piscataway, NJ). For denaturing gels, samples were diluted in SDS sample buffer without reducing agent, heated to 100°C for 1 min to avoid breakdown of polymers, and electrophoresed on 7.5% polyacrylamide gels (ExcelGel; Pharmacia Biotech). Proteins were transferred to nitrocellulose (Hybond ECL; Amersham Corp., Arlington Heights, IL), immunostained with rabbit anti–mouse α chain (Zymed Laboratories, Inc.) and detected with the ECL chemiluminescent system with horseradish peroxidase–conjugated secondary antiserum (Amersham Corp.).

Secretory IgA Collections. Freshly voided fecal samples were vacuum dried and extracted in PBS with 5% nonfat dry milk and protease inhibitors. Bile was aspirated from dilated gall bladders 24 h after bile duct ligation.

In Vitro pIgR-transfected Madin-Darby Canine Kidney Cell Experiments. Wild-type Madin-Darby canine kidney (MDCK) strain II cells or MDCK Strain II cells expressing the rabbit pIgR (20) were maintained in DME containing 10% fetal bovine serum and antibiotics. For transcytosis assays, cells were plated at confluent density onto 12-mm filter chambers (0.4-μm pore Transwells; Costar Corp., Cambridge, MA) and cultured for 4 d before use. Filters were then washed twice in serum-free DME and placed into 12-well plates containing 500 μl/well DME supplemented with serum (10%) from either wild-type or J chain–deficient mice. An equal volume of serum-free medium was added to the upper buffer chamber. The cultures were then incubated for 72 h at 37°C in 5% CO_2, after which samples of both apical and basolateral medium were removed from the filter chambers and stored at −20°C until IgA and IgG levels were measured by ELISA.

Results

The J Chain Gene Was Inactivated by Homologous Recombination in ES Cells. To inactivate the J chain gene in ES cells, a targeting vector was constructed using a 9.3-kb fragment encoding the entire murine J chain gene (Fig. 1 *A*). The gene was interrupted in exon 2, the first coding exon of the mature protein, by the neo^r. In addition, the *Herpes simplex* thymidine kinase gene was placed outside the region of homology to allow negative selection against random integration events. The linearized targeting construct was then introduced by electroporation into C1 cells, a 129/SvJ ES cell line (D. A. Connor, unpublished data). ES cells surviving G418, a neomycin analogue, and FIAU selection were screened for insertions in the J chain gene by Southern blot analysis using an external 5′ J chain genomic probe. ES cells containing the mutated J chain gene exhibited a novel 4.8-kb BamHI fragment due to the introduced neo^r sequences. Three independent targeted clones were identified among 51 neo^r FIAU^r clones analyzed. Cells from these three clones were then injected into C57Bl/6 blastocysts. One of these injected clones yielded four germline chimeric males. Heterozygous offspring of founder males were bred to produce mice homozygous for the J chain mutation (Jch$^{-/-}$). Mice heterozygous (Jch$^{+/-}$) and

homozygous for the J chain mutation were characterized by Southern blot analyses of tail DNA (Fig. 1 *B*).

To confirm that $Jch^{-/-}$ mice lacked the ability to express the J chain, Northern blot analyses of RNA from $Jch^{-/-}$ and wild-type ($Jch^{+/+}$) spleen cells were performed. $Jch^{+/+}$ mouse–derived splenocytes had easily detectable 1.3-kb J chain mRNA, whereas no J chain message was noted in splenocytes from the $Jch^{-/-}$ mice despite overloading the lane as judged by ethidium bromide staining and actin probe hybridization (Fig. 1 *C*). The inability of $Jch^{-/-}$ mice to produce J chain protein was documented by Western blot analysis using an anti–human J chain Ab that cross-reacts with mouse J chain. J chain protein was easily visualized in IgA hybridoma P1 E10 supernatant and in the serum of $Jch^{+/+}$ mice. However, no J chain protein was detectable in the serum of $Jch^{-/-}$ mice (Fig. 1 *D*).

J Chain–deficient Mice Have Elevated Serum IgA Levels. $Jch^{-/-}$ mice, maintained in a virus-free animal facility, have been observed through 12 mo of age and appear healthy and grossly normal compared with their wild-type littermates. FACS® analysis of splenocytes from $Jch^{-/-}$ mice using mAbs to μ chain, B220, CD3, CD4, CD8, and syndecan-1 (21), a murine plasma cell marker, revealed staining patterns indistinguishable from $Jch^{+/+}$ littermates (data not shown).

Serum immunoglobulin levels were examined by ELISA in 6–10-wk-old $Jch^{+/+}$ and $Jch^{-/-}$ mice. Measurement of serum IgM and IgG levels in 14 mice (7 $Jch^{+/+}$ and 7 $Jch^{-/-}$) revealed no significant differences between $Jch^{+/+}$ and $Jch^{-/-}$ animals (Table 1). In contrast, serum IgA levels in $Jch^{-/-}$ mice were >30 times greater than in $Jch^{+/+}$ mice (Table 1).

Table 1. *Serum Ig Levels in $Jch^{+/+}$ and $Jch^{-/-}$ Mice*

Ig	$Jch^{+/+}$	$Jch^{-/-}$	Significance
IgM	174 ± 56	148 ± 47	NS
IgG	1,132 ± 565	1,021 ± 338	NS
IgA	90 ± 53	2,929 ± 2,027	$p = 0.003$

Values listed are the mean ± SD ($n = 7$) reported in micrograms per milliliter.

The IgA Dimer:Monomer Ratio Is Altered in $Jch^{-/-}$ Serum. To distinguish between monomeric and dimeric IgA, sera from $Jch^{+/+}$ and $Jch^{-/-}$ mice were fractionated by PAGE. Serum samples were electrophoresed under both nondenaturing and denaturing conditions. IgA was detected by Western blotting with an anti–mouse α-chain Ab (Fig. 2). As predicted by the ELISA, dramatically more IgA was found in $Jch^{-/-}$ serum than $Jch^{+/+}$ serum. Monomeric, dimeric, and higher polymeric forms of IgA present in $Jch^{+/+}$ serum were also detected in $Jch^{-/-}$ serum (Fig. 2 *A*). Heating to 100°C for 4 min in the presence of dithiothreitol (DTT) reduced the dimeric forms to their component heavy chains (data not shown). Whereas the dimer/monomer ratio was, as previously reported (14), ~1:1 in wild-type mice, $Jch^{-/-}$ mice had at least 10 times more monomer than dimer by quantitative densitometry (Fig. 2 *A* and data not shown). Nonetheless, because of the elevated levels of serum IgA in the $Jch^{-/-}$ mice, the absolute amounts of dimer present in $Jch^{-/-}$ serum appeared comparable to if not somewhat greater than that in $Jch^{+/+}$

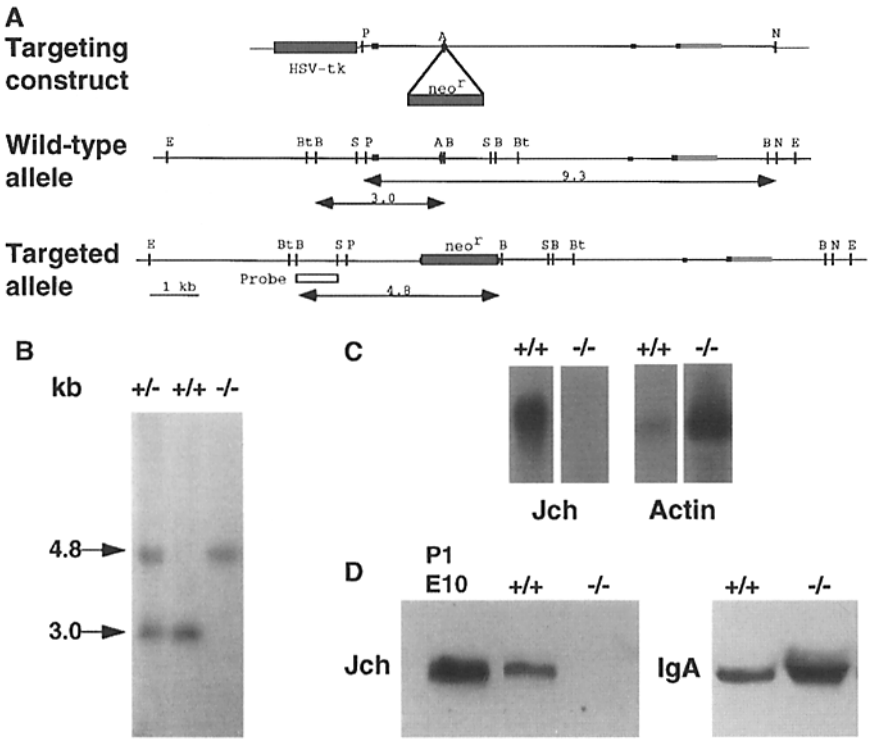

Figure 1. Disruption of the J chain gene by homologous recombination. (*A*) J chain gene targeting construct. *A*, Ava I; *Bt*, BstE2; *E*, EcoRI; *N*, NcoI; *P*, PstI. (*B*) Southern blot analysis of BamHI-digested tail DNA from offspring of a mating of two heterozygotes derived from a germline transmitting male chimera. Using the probe shown in *A*, mice with both J chain alleles disrupted exhibited only a 4.8-kb band, whereas wild-type animals exhibited a 3.0-kb band. Representative $Jch^{+/+}$, $Jch^{+/-}$, and $Jch^{-/-}$ mice are shown. (*C*) Northern blot analysis using J chain cDNA and murine actin probes of splenocyte RNA from $Jch^{+/+}$ and $Jch^{-/-}$ mice. (*D*) Western blot analysis of $Jch^{+/+}$ and $Jch^{-/-}$ serum and supernatant from an IgA hybridoma cell line P1 E10, used as a control. Serum samples were diluted 1:100 in sample buffer containing DTT, fractionated by SDS-PAGE on a 15% gel and immunoblotted with anti–human J chain Ab. The blot on the right shows the same two $Jch^{+/+}$ and $Jch^{-/-}$ lanes probed with an anti-α chain Ab.

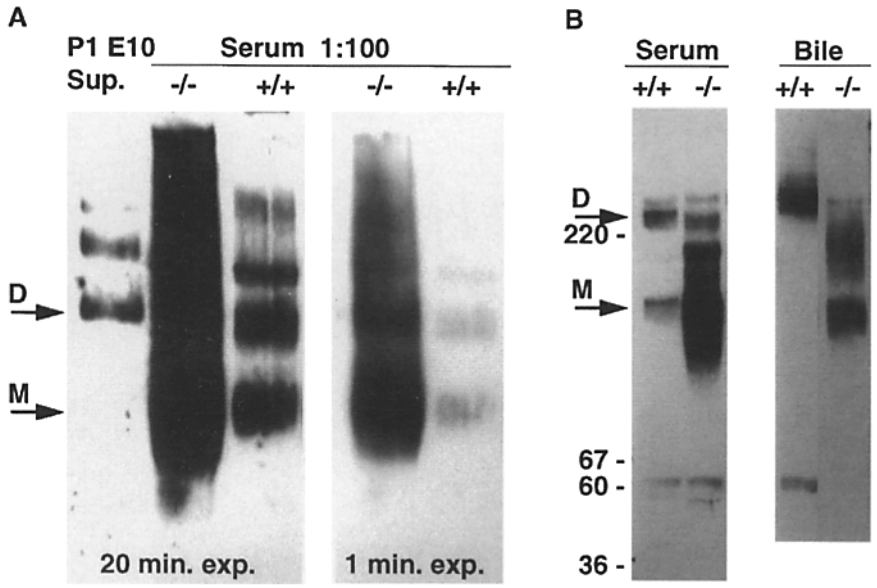

Figure 2. IgA in serum and bile samples from $Jch^{+/+}$ and $Jch^{-/-}$ mice immunostained with anti–mouse α-chain Ab. (*A*) Serum diluted 1:100 electrophoresed under nondenaturing, nonreducing conditions. Monomeric, dimeric, and higher polymer bands are seen. IgA hybridoma P1 E10 supernatant, which consists primarily of dimers and higher polymers, is included as a control. 20- and 1-min exposures of the $Jch^{-/-}$ and $Jch^{+/+}$ lanes are shown. Monomer (*M*) and dimer (*D*) bands are indicated. (*B*) Representative serum diluted 1:100 and bile diluted 1:2 electrophoresed under nonreducing conditions in the presence of SDS. Molecular mass markers in kilodaltons are shown at left.

mouse serum. We suggest that although IgA is able to dimerize in the absence of J chain, either dimerization is less efficient or J chain–deficient dimers are less stable than normal dimeric IgA.

J Chain–deficient IgA Is Not Transported Efficiently into Bile. To examine the ability of $Jch^{-/-}$ mice to secrete IgA into bile, we measured IgA levels in bile aspirated from dilated gall bladders 24 h after bile duct ligation. In addition, we measured fecal IgA levels, which are derived, at least in part, from bile. IgA levels in bile were 3.5 times lower in the $Jch^{-/-}$ mice than $Jch^{+/+}$ mice (Table 2). In addition, fecal IgA levels were profoundly decreased in $Jch^{-/-}$ mice (Table 2). Thus, these findings—elevated serum IgA with a corresponding decrease in bile and fecal IgA—suggested that $Jch^{-/-}$ mice have a defect in the hepatic transport of bloodstream-derived IgA into bile.

$Jch^{-/-}$ Bile Contains Nondimeric Forms of IgA Not Seen in $Jch^{+/+}$ Bile. Samples of serum and bile from $Jch^{-/-}$ and $Jch^{+/+}$ mice were then electrophoresed on nonreducing, SDS-containing gels and the transferred proteins were detected with an anti–mouse α-chain Ab. Although nearly all of the IgA present in $Jch^{+/+}$ bile was dimeric, studies of $Jch^{-/-}$ bile–derived IgA revealed very small amounts of dimer and prominent lower molecular weight bands (Fig. 2 *B*). The composition of these IgA-immunoreactive lower molecular weight bands is not yet defined.

Table 2. *IgA Levels Found at Different Sites in $Jch^{+/+}$ and $Jch^{-/-}$ Mice*

Site	$Jch^{+/+}$	$Jch^{-/-}$	Significance
Serum	137 ± 49	4,852 ± 1,580	$p < 0.01$
Feces	564 ± 286	11 ± 9	$p < 0.05$
Bile	212 ± 130	62 ± 5	$p < 0.05$

IgA levels are reported in micrograms per milliliter except those of fecal samples, which are micrograms per gram. Values listed are the mean ± SD ($n = 3$), except for the bile of $Jch^{-/-}$ mice ($n = 4$).

$Jch^{-/-}$ Serum–derived IgA Is Not Selectively Transported by pIgR in an In Vitro System. The hepatic pIgR transports serum-derived pIgA into bile (10–13). pIgA binds to the pIgR and is transported as a complex to the bile–canalicular surface, where the receptor is proteolytically cleaved, with a portion known as secretory component (SC) remaining associated with the pIg in bile. We hypothesized that the inefficient hepatic transport of IgA in $Jch^{-/-}$ mice was due to an inability of these receptors to transport J chain–deficient IgA. To examine the capability of the pIgR to transport J chain–deficient IgA, we used MDCK cells stably transfected with the pIgR cDNA to study transport of $Jch^{+/+}$ mouse–derived IgA versus $Jch^{-/-}$ mouse–derived IgA. Equivalent volumes of $Jch^{+/+}$ or $Jch^{-/-}$ serum were added to the basolateral surfaces of polarized monolayers of MDCK cells (wild-type or pIgR-expressing) grown on membranes in two-chambered vessels. After 72 h, supernatants from the apical chambers, which contained IgA transported across the MDCK monolayers, and supernatants from the basal chambers were collected. Supernatant levels of IgG, which is not transported by the pIgR, and IgA were then measured by ELISA (Fig. 3 *A*). As expected, $Jch^{+/+}$ mouse–derived IgA was selectively transported by the pIgR-expressing ($pIgR^+$) cells, with apical chamber IgA levels 22-fold higher than the apical chamber IgA levels of MDCK cells not expressing pIgR ($pIgR^-$). In contrast, similar amounts of $Jch^{-/-}$ mouse–derived IgA were noted in the apical chambers of $pIgR^-$ and $pIgR^+$ MDCK cells. In addition, the fraction of $Jch^{-/-}$ mouse–derived IgA transported by the $pIgR^+$ cells was similar to that of IgG transported by both $pIgR^+$ and $pIgR^-$ cells (Fig. 3 *B*). Addition of $Jch^{-/-}$ serum to basal chambers containing $Jch^{+/+}$ serum did not appear to inhibit transport of $Jch^{+/+}$

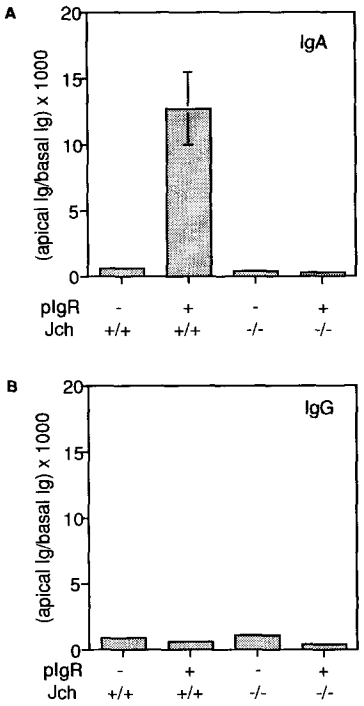

Figure 3. Ratio of apical to basal chamber levels of IgA (*A*) and IgG (*B*) collected 72 h after addition of $Jch^{+/+}$ or $Jch^{-/-}$ serum to either non-expressing ($pIgR^-$) or expressing ($pIgR^+$) MDCK cells. Values graphed are the mean ± SD (n = 3) from a representative experiment. SD for all but the IgA ratios for $Jch^{+/+}$ serum added to $pIgR^+$ cells are too small to show on graph.

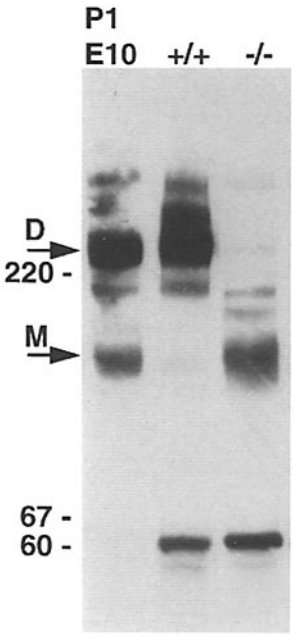

Figure 4. Structure of apical chamber $Jch^{+/+}$ and $Jch^{-/-}$ mouse–derived IgA transported by $pIgR^+$ MDCK cells. IgA hybridoma P1 E10 supernatant is included as a control. Representative samples of apical chamber media concentrated with microconcentrators (Microcon-30; Amicon Inc., Beverly, MA) were fractionated under nonreducing conditions in the presence of SDS and immunostained with an anti-α chain Ab. As noted with other IgA dimer–rabbit SC interactions (32), $Jch^{+/+}$ mouse–derived IgA dimers appear to associate with rabbit SC by predominantly noncovalent interactions, which disassociate in SDS. Monomer (*M*) and dimer (*D*) bands are indicated. Molecular mass markers in kilodaltons are shown at left.

mouse–derived IgA by the $pIgR^+$ MDCK cells (data not shown).

The pIgR binds dimeric IgA but not monomeric IgA (2, 7). Consequently, we examined the structure of the $Jch^{+/+}$ mouse–derived IgA and $Jch^{-/-}$ mouse–derived IgA transported by the $pIgR^+$ MDCK cells by fractionating apical chamber samples on nonreducing, SDS-containing gels and immunostaining with an anti-IgA Ab. Although nearly all of the $Jch^{+/+}$ mouse–derived IgA transported by $pIgR^+$ cells was dimeric, $Jch^{-/-}$ mouse–derived IgA in the apical chambers of $pIgR^+$ cells consisted primarily of nonpolymeric forms (Fig. 4). pIgA was still detectable at 72 h in basal chambers to which $Jch^{-/-}$ serum had been added (data not shown). The data above strongly suggested that the $Jch^{-/-}$ mouse–derived IgA was transported in these experiments by a nonreceptor-mediated transport mechanism, such as fluid phase transcytosis.

Discussion

We conclude that the J chain is not required for the secretion of IgM or dimeric IgA by B lymphocytes in vivo. However, our data indicate that although IgA dimers are produced in the absence of the J chain, either dimerization is less efficient or the J chain–deficient dimers are less stable than normal pIgA. We also conclude that the hepatic pIgR clearance of bloodstream IgA is impaired in $Jch^{-/-}$ mice.

The J chain is expressed in nearly all pIgA-producing cells in secretory glands and tissues, implying a physiologic importance in these cells (1, 2, 7). However, the J chain's role in IgA polymerization has remained ill defined. Previous work indicated that polymerization of IgA from its reduced subunits required the presence of the J chain (22, 23). In addition, Ma et al. (24) recently described transgenic plants expressing a hybrid immunoglobulin A-G heavy chain that appeared to require the J chain for dimerization. By contrast, human IgA myeloma proteins that lack the J chain or contain less than one J chain per polymer have been reported (5, 6, 8). Western blot analysis of serum from $Jch^{-/-}$ mice revealed high molecular weight IgA-immunoreactive bands migrating similar to IgA dimers in $Jch^{+/+}$ serum. Similar to wild-type IgA dimers, these high molecular weight forms reduced in the presence of DTT to their component heavy chains. However, whether these J chain–deficient dimers are correctly assembled, including in regard to their disulfide bonds, is not yet defined.

Our data suggest that the J chain is not necessary for dimer formation but is required for the normal IgA dimer/monomer ratio found in mouse serum. One possibility is that the addition of the J chain to dimeric IgA contributes significantly to the stability of the intermolecular disulfide bonds or important noncovalent interactions. The J chain's contribution to the stability of the dimeric structure may influence the efficiency of the polymerization process or promote dimer stability after secretion from the B cell. The J chain may also influence the stability of IgA in secretions. Bile samples from $Jch^{-/-}$ mice have lower molecular weight IgA-immunoreactive bands not seen in $Jch^{+/+}$ bile samples. The composition of these IgA-immunoreactive lower molecular weight bands remains to be determined. These bands may represent serum-derived dimer break-

down products or possibly monomeric IgA bound to other bile proteins. Depolymerization of pIgA after transport into bile also may account for some of the lower molecular weight forms, including monomer, seen in the bile in $Jch^{-/-}$ mice. Also monomeric IgA is believed to be more susceptible than SC-associated dimeric IgA to degradation in the intestinal tract (25). The J chain–deficient dimer may also be more susceptible to degradation in the intestinal tract, contributing to the very low fecal IgA levels found in $Jch^{-/-}$ mice.

Previous studies have demonstrated the importance of hepatic clearance of IgA in maintaining serum IgA homeostasis in rodents (10–15). For example, ligation of the bile duct in mice and rats is known to cause elevations in serum IgA (10, 14). Hepatic transport of IgA into bile may be an important mechanism for delivering IgA into the intestinal tract in rodents (15). This pathway is less relevant in humans, where the vast majority of intestinal IgA is produced in the submucosa and directly transported across the overlying intestinal epithelium. Transport of IgA into bile occurs in humans to a much lesser degree owing to restriction of pIgR expression to biliary duct epithelial cells (15). Although the elevated serum IgA levels found in $Jch^{-/-}$ mice could theoretically result from increased IgA production, we hypothesized that inefficient hepatic transport of J chain–deficient IgA accounted for this finding. The depleted bile and fecal IgA levels in $Jch^{-/-}$ mice compared with $Jch^{+/+}$ mice are consistent with this hypothesis.

The major receptor responsible for IgA transport into bile and mucosal secretions is the pIgR (2, 7, 10–13). The J chain's role in the binding of pIgA to the pIgR is unclear (2, 26). Brandtzaeg and Prydz (5, 6) reported that the ability of pIgA to bind to $pIgR^+$ epithelial cells, and SC was related to J chain content. However, Tomasi and Czerwinski (8) described pIgA myeloma proteins that appeared to lack the J chain but formed complexes with SC in vitro. Several studies have indicated that the J chain does not bind directly to SC (2). Studies of cleaved human secretory IgA indicated that J chain and SC are bound to different fragments of the α heavy chain and are not disulfide linked (27). Other studies have shown that SC forms disulfide bridges only with IgA and not with the J chain (28). Our studies revealed that MDCK cells transfected with pIgR were able to selectively transport $Jch^{+/+}$ mouse–derived IgA but not $Jch^{-/-}$ mouse–derived IgA. These data are consistent with the model that the J chain is necessary for the binding of pIgA to pIgR. One possibility is that the presence of the J chain in dimeric IgA influences the conformation of the α heavy chain domains and allows binding to pIgR and SC. Alternatively, J chain–deficient dimers are able to bind to pIgRs, but lack of the J chain leads to impairment in other aspects of the IgA transcytosis pathway. The observation that $Jch^{-/-}$ mouse–derived IgA does not appear to interfere with $Jch^{+/+}$ mouse–derived IgA transport by $pIgR^+$ MDCK cells (data not shown) argues, however, against this possibility. Of interest, unlike bile and fecal IgA levels, IgA levels are not depressed in other secretions in $Jch^{-/-}$ mice (Hendrickson, B. A., unpublished results). The role of pIgR in J chain–deficient IgA transport into these secretions is under investigation.

The pIgR transports dimeric IgA but not monomeric IgA (2, 7). Although $Jch^{-/-}$ mice have lower serum IgA dimer/monomer ratios than $Jch^{+/+}$ mice, comparable amounts of serum dimer are present and presumably available for transport in $Jch^{-/-}$ mice. Hence, deficiency of serum IgA dimer is an unlikely explanation for the low bile IgA levels in $Jch^{-/-}$ mice. Instead, an inability of hepatic pIgRs to transport the J chain–deficient dimer appears to be the preferable explanation for the hepatic IgA transport defect in $Jch^{-/-}$ mice. Numerous studies have shown the importance of pIgR in the transport of bloodstream-derived pIgA into bile (15, 26). Although the asialoglycoprotein receptor has also been implicated in serum IgA clearance, IgA internalized by this mechanism appears to be primarily targeted for lysosomal degradation (29, 30). Some evidence exists for IgA binding by hepatocytes, which appears to be unrelated to pIgRs or asialoglycoprotein receptors; however, these other putative receptors have not been ascribed a role in IgA transport into bile (7, 31). Thus examination of $pIgR^+$ MDCK cell transport of $Jch^{-/-}$ mouse–derived IgA is a relevant model for hepatic IgA transport in $Jch^{-/-}$ mice. This experimental system should be useful in delineating the steps that are impaired in pIgR transport of J chain–deficient IgA.

$Jch^{-/-}$ mice will be valuable tools in elucidating the mechanism of IgA dimer stabilization by the J chain and in the study of the IgA transport mechanisms in the liver and mucosal epithelium. In addition, IgA secretion into bile has been proposed to play a role in the immune protection of the biliary and upper gastrointestinal tracts and in the clearance of detrimental IgA–antigen complexes from the bloodstream (15). $Jch^{-/-}$ mice may provide a useful model for examining the relative importance of the biliary IgA secretory pathway in these processes.

We thank Dr. Marian Koshland for providing us with the J chain genomic clone and cDNA, and Dr. Merton Bernfield for the syndecan-1 antibody. We are indebted to Juanita Campos-Torres for her assistance with the FACS® analysis.

B. A. Hendrickson is supported by a National Institutes of Health (NIH) Physician Scientist Award. D. Kendall and M. R. Neutra are supported by NIH grant HD17557. J. G. Seidman, D. J. Ladd, and C. E. Seidman are supported by the Howard Hughes Medical Instiitute. J. E. Casanova is supported by NIH award

A132991. B. Corthesy is supported by SPP/Biotechnology grants 5002-38009 and 5002-34603 from the Swiss National Science Foundation.

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Received for publication 5 May 1995 and in revised form 19 June 1995.

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