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Lack of J Chain Inhibits the Transport of Gut IgA and Abrogates the Development of Intestinal Antitoxic Protection

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Recent publications have provided confusing information on the importance of the J chain for secretion of dimeric IgA at mucosal surfaces. Using J chain-deficient (J chain<sup>−/−</sup>) mice, we addressed whether a lack of J chain had any functional consequence for the ability to resist challenge with cholera toxin (CT) in intestinal loops. J chain<sup>−/−</sup> mice had normal levels of IgA plasma cells in the gut mucosa, and the Peyer’s patches exhibited normal IgA B cell differentiation and germinal center reactions. The total IgA levels in gut lavage were reduced by roughly 90% as compared with that in wild-type controls, while concomitantly serum IgA levels were significantly increased. Total serum IgM levels were depressed, whereas IgG concentrations were normal. Anti-CT IgA spot-forming cell frequencies in the gut lamina propria were normal. Anti-CT IgM concentrations were low in serum and gut lavage, whereas anti-CT IgG titers were unaltered. Challenge of small intestinal ligated loops with CT caused dramatic fluid accumulation in immunized J chain<sup>−/−</sup> mice, and only 20% protection was detected compared with unimmunized controls. In contrast, wild-type mice demonstrated 80% protection against CT challenge. Mice heterozygous for the J chain deletion exhibited intermediate gut lavage anti-CT IgA and intestinal protection levels, arguing for a J chain gene-dosage effect on the transport of secretory IgA. This study unequivocally demonstrates a direct relationship between mucosal transport of secretory SlgA and intestinal immune protection. The Journal of Immunology, 1999, 163: 913–919.

Mucosal surfaces constitute a first line of defense against microbial invasion of the body. Apart from the mucus layer, the barrier is maintained by the continuous production and transport of Abs, which are formed by plasma cells in the lamina propria (LP). The predominant isotype is IgA, whereas IgM and IgG Ab-producing cells are infrequently found at most mucosal surfaces. Of these three isotypes, IgA and IgM, but not IgG, are actively transported via the epithelial cell into the lumen. Therefore, intestinal secretions contain over 80% polymeric IgA, some polymeric IgM, but almost no IgG Abs. In response to infection or to immunization with Ag, specific Abs are formed, although even in the noninfected naive animal intestinal IgA formation is high, probably reflecting the constant stimulation of local immune responses by the commensal gut flora. Thus, the barrier functions of the mucosa appear to critically depend on an efficient transport system of dimeric IgA, and perhaps also of polymeric IgM.

This transport is mediated by uptake of dimeric IgA and polymeric IgM containing a J chain, which binds to the polymeric Ig receptor (plgR) expressed on the basolateral side of the epithelial cell. Thus, the plasma cell produces the J chain, which is a 15-kDa glycoprotein that covalently associates with disulfide linkages with polymeric IgA and IgM, but not to IgG, before secretion. After binding of the J chain Ig polymer to the plgR, the complex is transported through the epithelial cell to the luminal side, where the receptor is proteolytically cleaved off (2, 5). This results in the formation of secretory IgA (slgA), shown to be particularly resistant to proteolytic degradation (1, 2). Whether polymeric IgM at mucosal surfaces also has increased stability compared with serum IgM is less well known.

To investigate in vivo the role of the plgR pathway for the transport of IgA into the lumen, Hendrickson et al. recently developed a J chain-deficient mouse (6, 7). In their first report, the concentration of IgA in feces was dramatically lowered compared with wild-type (WT) mice. However, in a follow-up study, using a different detection system, IgA Abs, albeit monomeric forms, were present in normal concentrations at the mucosal surfaces in the intestine, mammary gland, and lung in J chain-deficient mice, suggesting that the J chain was not absolutely required for IgA transport into the lumen. This unexpected finding contrasted with the requirement for J chain in the hepatic transport of IgA found in these same mice (6). Although most previous studies have demonstrated a clear association between the presence of J chain and transport of IgA into the lumen (1, 2, 4, 5, 8), there are a few studies that support the view that IgA may reach the lumen through a J chain-independent plgR-mediated transport (2, 9, 10). The mechanism or the functional relevance of such a system has not been investigated.

Cholera toxin (CT) induces severe diarrhoeal disease (11). Following active oral immunization in humans and experimental animals, a high degree of antitoxic protection is induced (11, 12). Protection is thought to depend on the presence of intestinal slgA.
Abs (13–15), although some investigators have argued for other factors, such as IFN-γ, neuropeptides, or anti-secretory factors (ASF), to be responsible for the development of antitoxic protection (16–18).

We recently developed a mouse with an inactivated J chain and have reported on the systemic effects on IgM secretion of this gene deletion (19). In the present study, we addressed whether the J chain plays an essential role in the secretion and transport of intestinal anti-toxin IgA and if this process is associated with an increased resistance to CT challenge of small intestinal ligated loops in orally immunized mice.

Materials and Methods

Mice

WT (J chain+/+) mice or mice with homozygous (J chain−/−) or heterozygous (J chain+/−) deletions of the J chain (19) were bred at the Department of Cellular and Molecular Biology at Lund University (Lund, Sweden) and housed under pathogen-free conditions at the Department of Medical Microbiology and Immunology at the University of Göteborg (Göteborg, Sweden) before experimentation.

Immunization

Age- and sex-matched mice were given three oral immunizations 10 days apart with 10 μg of CT (List Biological Laboratories, Campbell, CA) in 6% (w/v) NaN3 in PBS by oral gavage followed by 0.5 mM PMSF and sodium azide to a final concentration of 1 mM and 0.001%, respectively. Mice were immunized by oral gavage 4 or 7 days after the final immunization as indicated. Control mice received PBS alone. Analysis of small intestinal antitoxic protection and systemic local anti-toxin Ab production were performed 4 or 7 days after the final immunization as indicated.

Intestinal lavage samples

Intestinal secretions for Ab determinations were collected by a method adopted from Elson et al. (21). Briefly, the small intestines were removed, rinsed in PBS to discard feces, and carefully injected with 3 ml of a proteinase inhibitor solution consisting of 0.1 mg/ml in PBS at 4°C overnight as described (23). Total IgA, IgM, or IgG determinations in intestinal content were quantified by ELISPOT assay.

ELISPOT assay

Individual cells secreting Igs, spot-forming cells (SFC), were determined by the ELISPOT technique essentially as described (24). Briefly, polystyrene petri dishes (Nunc; Nunc) were coated with 3 mg/ml of GM1 ganglioside (Sigma) in PBS at 4°C overnight, followed by an additional incubation at 4°C with 3 μg/ml CT (List Biological Laboratories) in PBS over night. For isolation of SFC, 96-well flat-bottom microtiter plates (Nunc) were coated with 5 μg/ml goat anti-mouse IgA, IgG, or IgM Abs (Southern Biotechnology Associates) in PBS at 4°C overnight. After washing with PBS, the petri dishes or the microtiter plates were blocked with 0.1% BSA in PBS at 30 min at 37°C. Lymphocytes at 4 × 105 in 400 μl medium were added to each petri dish or 103 lymphocytes in 150 μl medium were added to the top row in 96-well plates and then serially diluted 1:3 in corresponding subwells and sedimented by centrifugation for 3 min at 400 rpm. All dilutions were performed using Iscove’s medium (Seromod, Berlin, Germany) supplemented with 10% FCS (Sermoend, 5 × 10−3 M 2-ME Sigma), 1 mM L-glutamine, and 50 μg/ml gentamicin. Cells were incubated for 4 h in 37°C in 5% CO2. After incubation, each petri dish or well was washed out by repeated rinsing in PBS containing 0.05% Tween 20. Single LP anti-CT SFC were visualized by adding goat anti-mouse IgG primary Ab at 1/300 dilution (Cappel, Durham, NC) followed by HRP-conjugated rabbit anti-goat IgG secondary Abs (Dako, Carpinteria, CA) at 1/5000. For the isotype-specific SFCs, AP-conjugated Abs (Southern Biotechnology Associates) at 1/500 dilution were used. For the petri dishes, (HRP substrate) paranaphthylene diamine substrate (Sigma) in 1% agar-in-PBS solution was used, and for the 96-well plates (AP substrate) with 1 mg/ml 4-bromo-3-chloro-4-indolyl phosphate (Sigma) in agarose-in-water as described (24). The SFCs were counted under low magnification. Isolated LP or PP lymphocytes were pooled from two mice, and each pair was analyzed in duplicate petri dishes or triplicate wells plus one uncoated well as a control for unspecified binding. Values were expressed as mean SFC/105 lymphocytes ± SD of at least three pairs per group.

Ligated loop test

For evaluation of protection against CT-induced diarrhea/fluid loss, the method described by Lange and Holmgren was used (25). The abdomen was opened under light ether anesthesia, and a 6- to 8-cm loop was ligated in the middle part of the small intestine. CT (List Biological Laboratories), 2.5 μg in 0.2 ml of PBS, was injected into the loop, and the abdomen was closed. After 4 h, the mice were sacrificed, whereafter the loop with its fluid content was weighed and its length was determined. Unimmunized mice of all strains were equally sensitive to CT injected in ligated small intestinal loops. Values for fluid accumulation in the ligated loops, reflecting the degree of immune protection, were expressed as the weight-per-length ratio in mg/cm ± SD of five to seven mice per group.

Immunohistochemistry

Frozen sections (5 μm) from unimmunized or CT-immunized mice were prepared on microslides using a cryostat (model 1720; Leitz, Wetzlar, Germany). Slides were fixed in 4% paraformaldehyde in citrate buffer, and the reaction was read at 450 nm using a Titertek Multispec spectrophotometer (Flow Laboratories, Irving, U.K.). AP-labeled Abs were visualized using phosphatase-substrate tablets nitrophenyl-substrate (Sigma) at 1 mg/ml in ethanolamine buffer, pH 9.6, added to the wells, and the reaction was read at 405 nm in the Titertek Multispec spectrophotometer (Flow Laboratories). Antitoxin titers were defined as the interpolated OD reading giving rise to an absorbance of 0.4 above background, which consistently gave OD readings in the linear part of the curve. Titers were given in log10 means ± SD of five mice per group. Total IgA, IgM, or IgG in μg/ml was calculated from standard curves generated by serial dilutions of purified hybridoma IgA, IgM, or IgG (PharMingen, San Diego, CA) of known concentrations. The isotype-specific antisera were highly specific and did not cross-react with purified proteins of other isotypes.
IgM+ cells in the gut-associated lymphoid tissue, fixed sections were double labeled with FITC-conjugated peanut (Arachis hypogaea) hemagglutinin (PNA; Sigma) and Texas Red-conjugated anti-mouse IgA or IgM (Southern Biotechnologies Associates), both at 1/100 dilution (26). For detection of IgA plasma cells in LP, the sections were labeled with FITC-conjugated anti-IgA (Southern Biotechnologies Associates). The slides were mounted with fluorescent mounting media (Dako), and sections were evaluated and photographed using DAS Mikroskop (Leica Microscope System, Wetzlar, Germany).

Statistical analysis
We used Student’s t test for independent samples for analysis of significance.

Results
Distribution of total IgA and IgM in serum and intestinal lavage in J chain-deficient mice
Conflicting information regarding the relative distribution of total IgA and IgM in serum and gut mucosal secretions in J chain-deficient mice have recently been published (6, 7, 19). However, previous studies assessed mucosal IgA levels in fecal extracts or by applying wicks tampons directly to the mucosal surface of the small intestine (6, 7, 27), but a more reliable method is to collect intestinal lavage fluid from the entire small intestine using a buffer containing strong protease inhibitors (13, 14, 20, 21). Thus, we collected serum and gut lavage from WT mice or mice with a homozygous or heterozygous deletion of the J chain gene (19). We found that both J chain+/− and J chain−/− mice had significantly reduced levels of total IgA and IgM in gut lavage compared with WT mice (Fig. 1). In contrast, and in agreement with previous reports (6, 7), serum IgA levels were increased (Fig. 1) while serum IgM levels were dramatically reduced in J chain−/− and J chain+/− mice compared with WT mice (19). The mean IgM values of three experiments were 34 ± 16, 113 ± 74, and 236 ± 98 μg/ml, respectively. Intestinal total IgG levels were similar in J chain−/−, J chain+/−, and WT mice and roughly 300-fold lower than serum IgG levels in these mice (not shown). At the single-cell level, IgM-producing cells (SCF) were significantly fewer in both the PP and LP of J chain−/− than in WT mice (Table I). In contrast, the level of total IgA SCF were similar in PP and LP of J chain−/− and WT mice, indicating that the ability to develop IgA responses was unperturbed in J chain-deficient mice. This conclusion was supported further by immunohistochemical analysis of IgA B cell differentiation and maturation at inductive sites, i.e., PP, as well as at the LP-effector site in the intestinal mucosa (Fig. 2).

Table I. Total number of isotype-specific SFCs in freshly isolated lymphocytes from PP or LP of naive J chain-deficient or WT mice

<table>
<thead>
<tr>
<th>Isotype</th>
<th>PP</th>
<th>LP</th>
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<tbody>
<tr>
<td>IgA</td>
<td>WT</td>
<td>J chain−/−</td>
</tr>
<tr>
<td>73 ± 18</td>
<td>82 ± 17</td>
<td>2996 ± 902*</td>
</tr>
<tr>
<td>IgG</td>
<td>29 ± 10</td>
<td>31 ± 746</td>
</tr>
<tr>
<td>IgM</td>
<td>33 ± 9</td>
<td>6 ± 3</td>
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</table>

*Values for single Ab-producing cells using the ELISPOT technique are given as mean numbers of SFC/106 isolated lymphocytes ± SD of three pairs of J chain−/− mice compared with WT mice (not shown). Further, detection of anti-CT SFC responses in the LP of immunized mice demonstrated that the development of specific Ab-producing cells in the LP of J chain-deficient mice was unaltered compared with that in WT mice (Table II). Therefore, the J chain deficiency was not associated with a defect in the induction of anti-CT IgA immunity, but was clearly hampering the secretion of specific IgA into the intestinal lumen.

Lack of anti-toxic protection following oral immunization of J chain-deficient mice
Given that host resistance against CT-induced diarrhea requires the neutralization of toxin with specific sIgA at the mucosal surface (13–15, 20, 28), we investigated the consequence of an impaired pIgR-mediated transport for the ability to develop gut antitoxic protection following oral immunization with CT (13, 28). We found that, whereas WT mice demonstrated strong protection against CT challenge, the J chain-deficient mice were poorly protected (Fig. 5). Compared with unimmunized control animals, WT mice exhibited 80% protection while J chain−/− mice were only protected to 20%, indicating that the lack of IgA transport in J chain-deficient mice dramatically reduced the ability to neutralize luminal CT. Heterozygous J chain+/− mice showed intermediate protection, consistent with a gene-dosage effect in the ability to
develop intestinal antitoxic protection. These statistically significant results ($p < 0.05$) argue for a strong dependence on J chain-pIgR transport of sIgA across the intestinal epithelium for local protective immunity against CT.

Discussion

Here, we have demonstrated that functional mucosal immunity is highly dependent on an active transport of specific IgA Abs to the mucosal surfaces. The present investigation unequivocally supports the notion that the J chain is involved in this process and that in its absence strong Ag-specific IgA immunity in the LP of the mucosa cannot mediate protection against a toxin-induced diarrheal challenge. Also, we observed a gene-dosage effect of the J chain gene for intestinal antitoxic protection and IgA immunity in the heterozygous and WT mice, strongly supporting the idea that the J chain is directly linked to the mucosal secretion of Igs.

Furthermore, the presence of gut lavage IgG Abs, the isotype that does not bind J chain and, therefore, cannot associate with the

FIGURE 2. IgA B cell differentiation and maturation in the gut mucosa of J chain-deficient mice. Light level micrographs (magnification, $\times 20$) demonstrating the presence of IgA$^+$ cells in the LP and GC reactions in the PP from a naive J chain $^{-/-}$ (left) and a WT (right) mouse. Frozen sections were prepared and labeled with FITC-conjugated anti-IgA (upper panel) demonstrating IgA$^+$ cells in the gut LP, FITC-conjugated PNA plus TXRD-conjugated anti-IgA (middle panels) demonstrating double staining (yellow/orange) showing the colocalization of IgA$^+$ B cells to the PNA$^+$ GC in J chain $^{-/-}$ and WT mice in the PP, and FITC-conjugated PNA plus Texas Red-conjugated anti-IgM PNA (bottom panels) demonstrating GC (green) and membrane IgM$^+$ cells in the PP. These slides are representative sections from three separate experiments.
Ab levels were determined by ELISA. Values represent log_{10} titers of five mice per group with serum anti-CT IgA (open bars) and serum anti-CT IgM (filled bars). The higher concentration of anti-CT IgA in serum of J chain^{−/−} mice was statistically significant compared with heterozygous J chain^{+/−} and WT mice (p < 0.05).

FIGURE 3. Greatly augmented serum anti-CT IgA levels while anti-CT IgM concentrations were reduced in perorally immunized J chain-deficient mice. J chain^{−/−}, J chain^{+/−}, or WT mice were given three oral doses with CT (10 μg/dose) and sacrificed 8 days after the final immunization. Serum Ab levels were determined by ELISA. Values represent log_{10} titers ± SD of five mice per group with serum anti-CT IgA (open bars) and serum anti-CT IgM (filled bars). The higher concentration of anti-CT IgA in serum of J chain^{−/−} mice was statistically significant compared with heterozygous J chain^{+/−} and WT mice (p < 0.05).

pIgR, did not differ between the three strains, arguing that passive diffusion is unlikely to account for the translocation of protective IgGs into the lumen. If this had been the case, then the 10-fold increased serum IgA antitoxin levels detected in J chain^{−/−} mice following immunization should also have augmented the representation of gut lavage IgA and protection in mice lacking the J chain relative to those that have intact J chain production. Whether IgM antitoxin Abs have a protective function against CT cannot be answered by the present study, because, consistent with our previous findings, J chain-deficient mice appeared to have a perturbed IgM secretion (19). However, in a parallel study using IgA-deficient mice (29), we observed a similarly low level of protection, around 20%, despite a 10-fold compensatory increase in gut lavage anti-CT IgM levels compared with WT mice (N.Y.L., unpublished observation). Thus, the latter observation would argue against a functional protective role of gut polymeric IgM antitoxin Abs in host resistance against CT.

FIGURE 4. Impaired transport of specific IgA antitoxin into the gut lumen of perorally immunized J chain-deficient mice. J chain^{−/−}, heterozygous J chain^{+/−}, or WT mice were given three oral doses with CT (10 μg/dose) and sacrificed 8 days after the final immunization. Serum anti-CT IgG and gut lavage anti-CT IgA Ab levels were determined by ELISA. Values represent log_{10} titers ± SD of five mice per group with serum anti-CT IgG (filled bars) and gut lavage anti-CT IgA (open bars). The lower concentration of anti-CT IgG in gut lavage of J chain^{−/−} mice was statistically significant compared with heterozygous J chain^{+/−} and WT mice (p < 0.05). The anti-CT IgA values for J chain^{−/−} and heterozygous J chain^{+/−} mice were also significantly different from each other (p < 0.05).

The findings reported here are at variance with a previous study by Hendrickson et al., using a different strain of J chain-deficient mice (6, 7). Although we targeted exon 1, whereas Hendrickson et al., targeted exon 2, of the J chain gene, we have no reason to believe that the targeting strategy can explain the conflicting observations, because both approaches led to a failure to detect J chain gene transcripts (6, 19). Rather, we think that differences in the methods for detection of mucosal Abs and perhaps to some extent environmental factors or the recently discussed polymorphism in I129 mice (30) could have influenced the results. Although the neutralizing ability of monomeric IgA and resistance to proteolytic degradation is poor compared with that of sIgA (1, 2, 4), we do not think that the J chain-deficient mice that we have studied secreted monomeric or dimeric IgA (lacking J chain) into the lumen, as was suggested by the findings of Hendrickson et al. (7).

First, we detected very little IgA in the gut lavage, despite the fact that our ELISA was sufficiently sensitive to detect high levels of the predominantly monomeric IgA (6, 19) in serum of the J chain^{−/−} mice. It should be noted that J chain-deficient mice also have substantial levels of dimeric IgA (6, 7, 19), supporting our observation that dimerization of IgA in the absence of J chain is not sufficient for luminal transport. Secondly, our lavage method of collecting intestinal IgA is more representative and sensitive than the wicks tampon method used previously. Although both methods

### Table II. Unaltered LP antitoxin IgA responses to oral immunization with CT in J chain-deficient mice

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<tr>
<th>Mice</th>
<th>Antitoxin IgA SFC/10^6 LP Cells</th>
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<tr>
<td>WT</td>
<td>6050 ± 302</td>
</tr>
<tr>
<td>J chain^{−/−}</td>
<td>5916 ± 589</td>
</tr>
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</table>

* Mice were given three oral doses of CT (10 μg/dose) and sacrificed 6 days after the final immunization. Specific anti-CT-producing cells (SFC) were assayed by the ELISPOT method, and values are given as means ± SD. Three pairs of mice were analyzed in each group. This is one representative experiment of three.

FIGURE 5. Impaired antitoxic protection in gut mucosa following oral immunization with CT in J chain-deficient mice. We determined functional antitoxic protective immunity in gut-ligated loops of perorally immunized J chain^{−/−}, J chain^{+/−}, or WT mice. Three oral immunizations with 10 μg/dose of CT were given, and 7 days following the final dose mice were analyzed for antitoxic protection against CT-induced diarrhea by the ligated loop test. Fluid accumulation in response to challenge with CT, 2.5 μg/loop, was determined and compared with that observed in CT-challenged loops of unimmunized control mice. Values in mg/cm ± SD of five to seven mice in each group with immunized mice (open bars) and unimmunized control mice (filled bars). The results of J chain^{−/−}, J chain^{+/−}, and WT mice are all statistically significantly different from each other (p < 0.05). The data is representative of three identical experiments giving similar results.
exclude feces, the wicks detect Abs in only a limited region, while the lavage method collects IgA from the entire length of the small intestine (20, 21, 27). Moreover, it is difficult to rule out the possibility that the tampons establish a fluid diffusion gradient that may affect the barrier functions of the intestinal mucosa, thereby allowing the diffusion of IgA from the LP and serum into the tampons. Therefore, we believe that the lavage method is more accurate and provides highly reproducible measurements of luminal IgA. Although Hendrikson et al. used the lavage method for detection of IgA in bronchoalveolar fluid of J chain-/− mice (7), we think that the increased levels of IgA that they reported reflect the transudation of tissue and serum IgA into the alveolar lumen, similar to that seen in the genital tract (1, 4). Third, CT causes fluid loss after binding to the GM1 receptor on the epithelial cell (11, 12). If normal levels of surface dimeric anti-CT IgA had been present in immunized J chain-/− mice (7), we would have expected these Abs to confer a higher degree of protection, because the ability to neutralize toxin should not be a function of the presence of J chain or secretory component (1, 4, 13–15, 28).

Our study clearly demonstrates a strong association between the presence of specific IgA Abs in the gut lumen and protection against CT-induced fluid loss. This finding agrees well with previous studies using other models to show a direct relationship between gut mucosal transport of IgA and protection, as, e.g., the back-pack IgA-hybridoma model developed by Neutra and co-workers (15, 28). However, evidence has been presented to suggest that anti-toxic protection may not be mediated solely by anti-toxic Abs and may involve additional factors such as IFN-γ, or other cytokines, neuropeptides, or ASF, which were recently cloned and purified (16–18). However, the present results are difficult to reconcile with any major anti-toxic protective function of these alternative factors in vivo, a conclusion supported further by our demonstration that anti-toxic protection develops normally in IFN-γ receptor-deficient mice (31) and that also IgA-deficient and B cell-deficient mice fail to develop significant anti-toxic protection (N.Y.L., unpublished observation). Of course, our results with the J chain-deficient mice do not exclude a protective function of ASF or other factors against CT-induced diarrhea, although we do not know why the absence of the J chain in our model would have affected the level or function of, e.g., ASF (16). We did observe 20% protection in immunized J chain-/− mice. Whether this level of protection was conferred by the presence of low levels of luminal anti-CT IgA, lacking J chain, or these alternative factors is not known. However, orally immunized B cell-deficient mice exhibit no protection at all against CT challenge (N.Y.L., unpublished observation). Also, we find it highly unlikely that there would be any connection between the neuroepitopes or ASF and the J chain, or for that matter a dependence on epithelial transcytosis of local dimeric IgA Abs and these alternative protective factors. Further analyses are required to delineate the role of these factors in intestinal anti-toxic protection.

Consistent with our previous report on the J chain-deficient mouse, we observed low levels of IgM Abs (19). Not only did we find decreased levels of IgM in gut lavage, we also had low serum IgM concentrations and 5-fold reduced total IgM SFC in the gut LP as compared with WT mice. Therefore, the low gut IgM levels in the J chain-/− mice seem to be an effect of poor plasma cell differentiation, rather than an effect of impaired plgR transport. Thus, this maturational defect appears to affect IgM B cells selectively, as both IgG and IgA production, including gut anti-CT IgA SFC, were normal. This is an intriguing finding, demonstrating that the production of J chain is not required for terminal maturation of IgA plasma cells in the gut. Because almost all IgA- or IgG-producing cells in human gut LP express J chain and only few cells of these isotypes produce J chains in peripheral lymph nodes, Brandtzaeg and coworkers have speculated that coexpression of J chain would constitute a maturational requirement for mucosal B cell development (1). However, the data from J chain-deficient mice does not appear to support such a notion (7, 19). At present, we do not have an explanation for why coexpression of J chain is selectively more important for IgM plasma cell differentiation. Potentially, the J chain is critical in the assembly and secretion of polymeric IgM (32). Alternatively, it plays no role in terminal plasma cell maturation; rather, it may be that, in the absence of J chain, the secreted form of IgM is highly unstable and is rapidly degraded in serum and tissue. However, the work of many investigators has indicated that the J chain is not critical for production of polymeric IgM, including hexameric IgM, which is the most complement-activating form of IgM (1, 33). In our previous study, we found lower concentrations of both hexameric and monomeric forms of IgM in serum of J chain-/− mice, and serum from these mice was a significantly poorer activator of complement compared with serum from WT mice, indicating that there are also qualitative differences (19). Further studies will be undertaken in this model to address the role of the J chain for the maturation of IgM plasma cells.

References


