IgA production in MHC class II-deficient mice is primarily a function of B-1a cells

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Keywords: cholera toxin, intestine, mucosal immunity, Th

Abstract

Mice deficient in MHC class II expression (C2d mice) do not make antibody to protein antigens administered systemically, but their ability to produce IgA antibody to antigen administered at mucosal sites has not been described. We investigated IgA production by C2d mice and their IgA antibody response to antigen given orally. Young C2d mice had normal amounts of serum IgA, intestinal-secreted IgA and normal numbers of intestinal IgA plasma cells, compared to control C57BL/6 mice. IgA production by C2d mice increased with age. Following oral immunization with cholera toxin, C57BL/6 mice responded with IgA and IgG antibody, and had increased numbers of IgA plasma cells, but C2d mice gave no response. The Peyer’s patch and mesenteric lymph node tissues of C2d mice contained very few CD4-expressing T cells. Thus, C2d mice have no typical mucosal CD4 Th cells and cannot respond to a strong oral immunogen, yet they still produced and secreted IgA. We hypothesized that B-1 lymphocytes could provide a source of IgA independent of antigen-specific T cell help. Young C2d mice had normal numbers of peritoneal B-1a cells and their frequency increased with age. To test the role of these B-1a cells, we bred C2d mice to obtain mice that had no MHC class II expression and expressed the Xid gene that confers deficiency in B-1a cells. These double-deficient mice had 10-fold less serum and secreted IgA than all other F2 littermates. We conclude that B-1a cells are essential for the majority of IgA production in C2d mice. Thus, the C2d mouse may provide a useful tool for analysis of the role of intestinal IgA provided by B-1a cells.

Introduction

IgA antibody responses have largely been studied in the context of oral immunization with protein antigens or particulate antigens that require CD4 T cells to provide help in an MHC class II-restricted manner. Naive B cells in Peyer’s patches are considered the precursor pool for plasma cells that eventually locate in the intestinal lamina propria and produce polymeric IgA, suitable for transport through the intestinal epithelium to the external lumen (reviewed in 1–3). Much of intestinal IgA has been attributed to responses by typical B cells (i.e. B-2 cells) that require cognate interaction with CD4 T cells. Thus, the intestinal IgA response has been studied primarily as a mechanism dependent on CD4 T cells. However, several years ago Kroese et al. provided strong evidence that peritoneal B-1 cells can populate the intestinal track of mice with plasma cells that produce IgA (4,5). B-1 cells are known to respond to certain types of antigen without T cell help and often produce antibody to bacterial or autoantigens (6,7). Recently B-1 cell transfer studies in SCID mice have indicated that IgA is produced and secreted by these cells, coincident with the finding of IgA bound to the surface of the normal cecal flora of the SCID mice (8,9). Thus, B-1 cells can provide some production of intestinal IgA and some of this IgA can interact specifically with intestinal flora, but the relative contribution of B-1 and typical B-2 cells to the total IgA in normal mice remains uncertain (10). In addition, it is still unclear whether IgA is produced by B-1a or B-1b subtypes (8).

Mice deficient in MHC class II expression were produced by two separate laboratories several years ago (11,12). These mice have very few peripheral T cells that express CD4, do not make antibody response to T cell-dependent antigens (e.g. proteins) given parenterally, but continue to make antibodies to T cell-independent antigens (e.g. TNP-lipopolysaccharide and α1,6-dextran) (11,13). Both B-1 and B-2 cells were found in young class II-deficient (C2d) mice similar to control C57BL/6 mice (13). In addition, C2d splenic (mostly B-2) cells...
respond to typical proliferative and activation stimuli in vitro, but not to stimuli that require antigen presentation by MHC II, including secretion of IgA along with IgG subclasses (13). Circulating Ig classes in C2d mice are nearly normal except for very depressed IgG1 levels and perhaps elevated IgM (11,13). A single report on serum IgA indicated that young C2d mice had normal levels (13). It is clear that the absence of MHC class II expression renders mice incapable of generating antibody response to systemically administered antigens that require cognate T cell help, but otherwise does not affect the development of either the B-1 or B-2 cell lineages. The observation that C2d mice have B-1 cells leaves open the potential for their contribution to IgA production. The CBA/N (Xid) mouse is deficient in production of B-1a cells (14,15). We therefore carefully characterized the intestinal IgA production in C2d mice and their response to intestinal antigen exposure, and then bred C2d mice with Xid mice in order to determine to what extent B-1a cells contribute to the total IgA production by C2d mice.

**Methods**

**Mice, breeding, immunizations, and sampling**

C2d mice were obtained from GenPharm (Mountain View, CA) and were bred at the McMaster Central Animal Facility, under barrier specific pathogen-free conditions. The genetic deletion (class II I-Aβ) together with the natural mutation on the I-Eβ chain was eventually expressed on the C57BL/6 mouse background, by successive backcross breeding (12). Female C57BL/6 and CBA/N mice were obtained from Jackson Laboratories (Bar Harbor, ME), at 5–7 weeks of age, and maintained in clean standard housing facility (sterile food and water and filtered cage tops). For immunization and age studies, C2d mice were removed from the barrier specific pathogen-free facility at 6 weeks of age and placed in the same standard housing as C57BL/6 mice, at least 2 weeks prior to use and for the remaining period of the experiment. For breeding of F2 generation mice, male C2d mice were first mated with female CBA/N mice to generate a mixed phenotype (Xid) mouse is deficient in production of B-1a cells (14,15). A single report on serum IgA indicated that young C2d mice had normal levels (13). It is clear that the absence of MHC class II expression renders mice incapable of generating antibody response to systemically administered antigens that require cognate T cell help, but otherwise does not affect the development of either the B-1 or B-2 cell lineages. The observation that C2d mice have B-1 cells leaves open the potential for their contribution to IgA production. The CBA/N (Xid) mouse is deficient in production of B-1a cells (14,15). We therefore carefully characterized the intestinal IgA production in C2d mice and their response to intestinal antigen exposure, and then bred C2d mice with Xid mice in order to determine to what extent B-1a cells contribute to the total IgA production by C2d mice.

**Immunohistology**

Sections of jejunal tissue (0.5 cm) were imbedded in Tissue-Tek (OCT) compound (Miles, Elkhart, IN) and then snap frozen in liquid nitrogen. Cryostat sections (5 µm) were cut, fixed with acetone, re-hydrated, blocked with 1% BSA in TBS and incubated for 30 min at 21°C with 5 µg/ml of affinity-purified, biotin–anti-IgA antibody (Southern Biotechonology, Birmingham AL). After rinsing with TBS, dilute horseradish peroxidase–streptavidin (Sigma, St Louis, MO) was then applied to each section followed by washing and 3-amino-9-ethyl-carbazole (AEC) substrate (Sigma). The reaction was stopped by washing with water. Sections were counter-stained with hematoxylin to reveal nuclei and tissue morphology. Sections were then examined under a ×20 objective, using an ocular micrometer to measure the length of villi. Only full-length villi were examined. All lamina propria nuclei were counted within a defined length of each villus. Nuclei associated with dark red (AEC) cytoplasmic staining were scored as IgA plasma cells. Plasma cell counts were then expressed per length of villi or as a percentage of the total lamina propria nuclei.

**ELISA**

IgA and IgG subclass antibodies reactive with CT were measured by solid-phase ELISA methods described previously, using biotinylated anti-IgA and anti-IgG subclass antibodies (Southern Biotechnology), streptavidin–alkaline phosphatase, and CT bound to microtiter wells (16,17). Total IgA, IgG1 and IgG2a in serum and IgA in gut washings were measured using sandwich-type ELISA. Matched pairs of unlabeled and biotinylated anti-Ig reagents were purchased (Southern Biotechnology), and streptavidin–alkaline phosphatase (Sigma) used for enzymatic detection. Unlabeled antibodies were coated on the wells at a final concentration of 2 µg/ml and biotinylated antibodies were diluted to 0.5 µg/ml for use. Purified mouse mAb or myeloma proteins of appropriate isotypes were available in our laboratory and were used to produce standard curves to measure the amount of each isotype (µg/ml) in the serum of the mice.

**Flow cytometry**

B-1a peritoneal cells were identified by staining techniques similar to previous reports (6) using goat anti-mouse IgM–phycoerythrin (PE) (Southern Biotechnology) and anti-CD5–FITC (clone S3-7.3) conjugate (PharMingen, San Diego, CA). Anti-CD8β–PE (clone S3-5.8) and anti-CD4–FITC (clone GK1.5) were both purchased from PharMingen. The allotypic
anti-mouse class II MHC mAb, clone 10-2.16 (18), that reacts with I-A^k, was produced in ascites, isolated by ammonium sulfate precipitation and HPLC size exclusion chromatography or on Protein A–Sepharose (Pharmacia, Uppsala, Sweden) and labeled with FITC (Sigma) in our laboratory. All antibodies were titrated for optimal binding, including those purchased from commercial suppliers. For all staining protocols, cells were pre-incubated with 5 μg of rat anti-mouse FcyRII/III mAb, clone 2.4G2 (19), to prevent Fc-mediated binding to B cells, followed by labeled antibodies, with incubation in 1% BSA/PBS at 4°C, for 30 min. Reaction volumes were 100 μl. Cells were washed and fixed with 1% paraformaldehyde in PBS, and analyzed on a FACScan instrument (Becton Dickinson, San Jose, CA). The FACScan was calibrated daily with CalibrE beads (Becton Dickinson). Data were acquired for a minimum of 10,000 events accumulated in a light scatter region defined for lymphocytes. Analysis was performed by Lysys II software (Becton Dickinson). Two-parameter plots used for frequency data were either dot-plots or 50% log probability plots as shown on the figures. All data were gated on a lymphocyte forward versus side scatter gate, prior to display in two-parameter histograms.

Statistics
Comparison between groups of ELISA or immunohistologic data was performed using the Student’s t-test. Both the t-test and the \( \chi^2 \)-test for frequency difference were performed using the MiniTab statistical software (MiniTab, State College, PA).

Results
Small intestinal IgA production in C2d mice
In order to first examine IgA production in C2d mice, the total levels of serum IgA were measured in 8- to 12-week-old C2d and C57BL/6 control mice. Data presented in Table 1 demonstrate the young C2d mice have considerable levels of serum IgA, at least as much as age-matched control mice. These data on serum IgA are consistent with the results from other laboratories (13). In contrast, a 9-fold reduction of IgG1 was found in C2d serum, but a more modest 3-fold reduction in IgG2a, also consistent with previous findings (11,13). In the mouse, secreted IgA is more reflective of local IgA production in the intestine, than serum IgA. Samples taken from washings of the small intestine of young C2d mice contain on average 3-fold higher amounts of IgA, compared to control age-matched C57/BL6 mice. Older C2d mice (9–15 months) continued to maintain very high levels of serum and secreted IgA (Table 1). To confirm that the large amount of secreted IgA is likely produced from local IgA plasma cells, we counted those plasma cells, staining by immuno-enzymatic techniques, in frozen tissue sections of small intestine. Plasma cells were counted and the number expressed per standard length of villus or as a percentage of the total nucleated cells in the lamina propria in examined full length villi (Table 2). By either criterion young C2d mice had similar numbers of IgA plasma cells to those in C57BL/6 mice. It was clear that young C2d mice have no deficiency in total IgA production or secretion in the small intestine and that IgA production was maintained until the mice were quite old.

Table 1. IgA production in C2d mice compared to C57BL/6 mice

<table>
<thead>
<tr>
<th>Ig</th>
<th>C2d (2–3 months)</th>
<th>Old C2d (9–15 months)</th>
<th>C57BL/6 (2–3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ig (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG1</td>
<td>77 (27)a</td>
<td>ND</td>
<td>712 (127)</td>
</tr>
<tr>
<td>IgG2a</td>
<td>285 (91)a</td>
<td>ND</td>
<td>880 (189)</td>
</tr>
<tr>
<td>IgA</td>
<td>132 (30)</td>
<td>402 (230)</td>
<td>81 (10)</td>
</tr>
<tr>
<td>Intestinal secreted (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>23 (8)a</td>
<td>37 (22)a</td>
<td>8 (2)</td>
</tr>
</tbody>
</table>

\( a \) Statistical significance ( \( p < 0.01 \)) difference from C57/BL6, for \( n = 6 \), using Student’s t-test comparison. ND, not determined

Lack of specific IgA antibody response to intestinal antigen in C2d mice
CT is a potent immunogen and intestinal adjuvant in mice, stimulating strong IgA responses (20–22). We immunized C2d and C57BL/6 mice orally with 10 μg of CT, and examined both IgA plasma cell numbers in the small intestine and the amount of IgA anti-CT antibody in serum. In normal C57BL/6 mice, a 3- to 4-fold increase in IgA plasma cell numbers were noted following oral CT immunization (Table 2). Only a modest increase in IgA plasma cells was found in C2d mice after CT immunization, but that number of plasma cells was not statistically different from untreated C2d mice. C57BL/6 mice also produced substantial titers of serum IgA anti-CT antibody along with some antibody in the IgG subclasses, particularly IgG1 (Fig. 1). No IgA or IgG subclass, anti-CT antibody was detected in the serum of C2d mice immunized with CT. Thus, C2d mice could not mount a mucosal IgA response to the strongest oral immunogen yet described. Additional studies (manuscript in preparation) examine the ability of C2d mice to clear a primary infection with Giardia muris, a common intestinal protozoan parasite in mice. Clearance of the parasite is dependent on both the antibody response (especially IgA) to parasite antigens and the function of CD4 T cells (23,24). We observed no antibody production to G. muris antigens and development of a chronic primary infection in C2d mice. These results clearly indicate that C2d mice cannot produce IgA antibody specific for protein antigens administered orally or against antigens from a protozoan parasite that colonizes the small intestine.

CD4 T cell deficiency and altered Peyer’s patch cellularity in C2d mice
C2d mice have been shown to have profound deficiency in the number and function of mature CD4 T cells, in systemic lymphoid tissues (11,13). In addition, others have examined subsets of T cells within the epithelial compartment of the C2d intestine (25,26). We therefore examined CD4 cells in the Peyer’s patches and mesenteric lymph nodes (MLN) that are the key tissues in induction and propagation of IgA responses to thymic-dependent antigens. For comparison, proportions of CD8 cells (CD8β expression) and IgM-
B-1a cells in C2d mice

The normal levels of total IgA, in the context of an inability to respond with IgA antibody to antigens that require T cell help, poses a clear question as to the origin of the IgA-secreting cells in the intestine of C2d mice. Recent work has clearly shown the B-1 cells can contribute to the total IgA secretion in the intestine (4,5). We examined C2d mice for the presence of B-1a cells in the peritoneal cavity and in intestinal tissues, comparing them to normal C57BL/6 mice. Figure 3 indicates...
that young C2d mice have normal proportions of B-1a cells (defined as CD5<sup>low</sup>, IgM<sup>+</sup> cells) in their peritoneal cavity, in agreement with the previous report of others (11,13). Older C2d mice show increased proportions of B-1a lymphocytes, with some 9-month-old mice having a large majority of their peritoneal lymphocytes of the B-1a phenotype. Further analysis indicated that young (2–3 month old) C2d mice had near normal total numbers of peritoneal B-1a cells (6.3 ± 0.3 × 10<sup>4</sup> per mouse versus C57BL/6 that had 3.9 ± 1.6 × 10<sup>4</sup> per mouse, n = 4). Peritoneal B-1a cells increased in number with age in C2d mice (25.1 ± 1.5 × 10<sup>4</sup> per mouse at 5 months and 16.3 ± 5.6 × 10<sup>4</sup> per mouse at 9–11 months of age).

Low IgA production in F<sub>2</sub> mice that expressed Xid and were deficient in class II MHC

To test if B-1a cells contribute the majority of IgA in C2d mice, we chose to breed C2d mice with mice expressing the Xid gene and then interbreed the F<sub>1</sub> progeny. CBA/N mice express the Xid gene and have barely detectable numbers of peritoneal B-1a cells as defined by the IgM<sup>+</sup> CD5<sup>low</sup> phenotype (14,27). Male C2d mice were bred with female CBA/N (Xid) mice, so that all F<sub>1</sub> mice male mice were Xid, increasing the frequency of Xid among the F<sub>2</sub> progeny. Interbreeding of F<sub>1</sub> mice would then predict that one-eighth of mice (male or female) would express both Xid and have MHC class II deficient. If the B-1a cells normally present in C2d mice were responsible for their IgA production, then loss of B-1a cells concomitant with MHC class II deficiency should render mice deficient in IgA production.

We examined all F<sub>2</sub> mice for the presence or absence of the class II MHC molecule I-A<sup>k</sup> (expressed by CBA/N mice) on their peripheral blood cells, using flow cytometry. This easily distinguished ~25% of the mice as C2d (Fig. 4A). Mice expressing Xid were distinguished by their low numbers of peritoneal cells with B-1a phenotype (IgM<sup>+</sup>, CD5<sup>low</sup>), using flow cytometry (Fig. 4B). Analysis of B-1a cell data from all 51 of the F<sub>2</sub> mice indicated a bimodal distribution, with ~50% of the mice having on average 4 times lower percentage of B-1a cells than the other mice (Fig. 4B). This was consistent with the prediction that 50% of the F<sub>2</sub> mice would exhibit the Xid defect by random chromosome assortment. We therefore classified the F<sub>2</sub> mice with <14% peritoneal B-1a cells as Xid mice. Mice scored as Xid also had substantially lower numbers of total peritoneal B-1a cells (17.1 ± 9.6 × 10<sup>4</sup> cells/mouse) compared to non-Xid mice (76.1 ± 38.9 × 10<sup>4</sup> cells/mouse). Using the two phenotypic markers (low percentage of B-1a cells and absence of MHC class II on peripheral blood lymphocytes), four groups of F<sub>2</sub> mice could be identified. Table 4 shows the frequency of the four groups of mice, identified by phenotype and compared to the expected frequency. The χ<sup>2</sup> test of comparison showed no significant difference in observed versus expected frequencies. Thus, the F<sub>2</sub> breeding yielded the expected distribution of normal, single- and double-deficient mice.

Comparison of serum and gut wash IgA among the four different groups of F<sub>2</sub> mice is shown in Fig. 5. F<sub>2</sub> mice that had no class II MHC and had low numbers of peritoneal B-1a cells, had very low levels of serum and intestinal IgA, ~1/10 of the amount of all other F<sub>2</sub> mice. Thus, the lack of MHC class II expression and reduction in B-1a cells resulted in very low amounts of IgA production, whereas mice that only had MHC class II deficiency or B-1 cell deficiency had near normal levels. This clearly indicates that IgA production in C2d mice is largely reduced if the Xid gene is also present and that the loss of B-1a cells correlates with loss of IgA.

Table 3. Lymphocyte subsets in Peyer’s patch, MLN and spleen of C2d mice

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>CD4 T cells (%)</th>
<th>CD8&lt;sup&gt;β&lt;/sup&gt; T cells (%)</th>
<th>IgM&lt;sup&gt;+&lt;/sup&gt; B cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td>1.4 (0.2)</td>
<td>9.4 (1.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.4 (1.7)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MLN</td>
<td>2.3 (0.1)</td>
<td>38.2 (4.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.8 (2.6)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.5 (0.3)</td>
<td>14.5 (0.6)</td>
<td>56.1 (3.8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>22.8 (3.9)</td>
<td>3.8 (0.7)</td>
<td>28.9 (1.3)</td>
</tr>
<tr>
<td></td>
<td>29.9 (2.0)</td>
<td>27.1 (4.2)</td>
<td>18.7 (1.9)</td>
</tr>
<tr>
<td></td>
<td>15.4 (1.1)</td>
<td>19.1 (1.1)</td>
<td>42.8 (2.0)</td>
</tr>
</tbody>
</table>

Values are mean (SEM) for n = 4 mice. Significant reduced percentages of CD4 T cells in C2d (bold type) versus C57BL/6 (P < 0.01). Significant increases in CD8<sup>β</sup> and IgM cells were also noted in some cases (*P < 0.05). Background stain was on average <0.3% for CD4, CD8 and IgM, in all tissues. Percentages are based on cells within the lymphocyte light scatter gate.
B-1a cells and IgA antibody in C2d mice

Fig. 4. Distribution of class II deficiency and Xid phenotype among F₂ progeny of F₁ CBA/N×C2d matings. (A) The flow cytometric testing of F₂ mice, where 25.5% (13 of 51) of the mice (shaded bar) had no detectable MHC class II cells in peripheral blood. (B) The bimodal population distribution for the percentage of peritoneal B-1 phenotype cells. The left-hand population (solid bars) that represented 49% (25 of 51) of all F₂ mice had a mean of 4.9% peritoneal B-1 cells compared to the right-hand population (hatched bars) with a mean of 21.9% B-1 cells.

Table 4. Frequency of Xid, C2d and mixed phenotype mice in F₂ progeny from mating F₁ mice generated by the mating of female CBA/N and male C2d mice

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Observed</th>
<th>Predicted</th>
</tr>
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<tbody>
<tr>
<td>Xid, C2d</td>
<td>6/51</td>
<td>1/8</td>
</tr>
<tr>
<td>C2d only</td>
<td>7/51</td>
<td>1/8</td>
</tr>
<tr>
<td>Xid only</td>
<td>19/51</td>
<td>3/8</td>
</tr>
<tr>
<td>Normal phenotype</td>
<td>19/51</td>
<td>3/8</td>
</tr>
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</table>

Discussion

Our results show that C2d mice cannot make any antibody response to CT, one of the strongest of oral immunogens in rodents (20–22). The MHC class II restriction of both the T cell and antibody response to CT is well described (28,29).

Neither serum IgG nor secreted IgA antibody were observed after oral immunization of C2d mice with CT. This contrasts somewhat the results of similar experiments performed with mice that cannot express the CD4 molecule (30). Those mice have IgA plasma cells and B cells in their intestinal tract, and secrete IgA. When CD4-deficient mice were immunized orally with CT they did not make intestinal IgA antibody, but were able to produce serum IgG antibody to CT. Thus, the CD4 molecule appeared essential to production of local anti-CT IgA responses, but systemic IgG responses could still be produced, presumably by CD4null T cells that were MHC class II restricted and could help B-2 cells produce that antibody (30). The lack of IgG antibody response from C2d mice must therefore reflect the functional absence of any T cell help that is MHC class II restricted.

Considerable experimental evidence has detailed the contribution of B-1 cells to intestinal IgA production (4,8,10), although the precise roles of B-1a and B-1b cells have not been worked out (8). The most critical evidence concerns the accumulation of donor IgA plasma cells in irradiated mice that received peritoneal cells from Ig allotype congenic mice (4) and the similar observation with peritoneal cells given to SCID mice (8,9). Additional evidence has been derived from observation of IgA plasma cell development in µ,κ transgenic mice, which have a small population of B-1 cells bearing exclusively endogenous IgM molecules (5). The IgA produced by the intestinal plasma cells in the µ,κ transgenic mice is also from endogenous genes, so that the IgA is most likely produced by the B-1 cells. A reciprocal example is motheaten mouse (me/mev) that has a defect in B-2 cell maturation, but still produces large quantities of IgA in its short life and has high numbers of B-1 cells (31). So there is clear evidence...
that B-1 cells can seed the mouse lamina propria with cells that develop into IgA-producing plasma cells, but the contribution of B-1 cells to the total plasma cell pool in the normal mouse or in humans is still not known nor are the mechanisms and circumstances under which B-1 cells switch to IgA-producing cells in vivo.

Our data show clearly that C2d mice produce IgA, have lamina propria plasma cells and secrete IgA into the intestinal lumen. IgA production and intestinal secretion increase with age, and at the same time C2d mice maintain a large number of B-1a cells in their peritoneal cavities. The CBA/N mouse also has large quantities of IgA and can respond to T cell-dependent antigens, including those provided by intestinal parasites (32). Thus, neither the absence of B-1 cells (CBA/N) nor the absence of cognate interactions between B cells and CD4 T cells (C2d) results in complete loss of IgA production. In fact, in both cases total IgA production is near normal. The two models may reflect compensation mechanisms for IgA production mediated through the reciprocal B cell types, each of which remains functional in either of the mice. By breeding F2 mice with a mixed genetic background from CBA/N and C2d mice, we determined that ~90% of the IgA made in the absence of MHC class II molecules is eliminated by the expression of the Xid phenotype. This reduction in IgA was coincident with a 4-fold drop in the population of mice that had the fewest B-1a cells was scored as Xid. However, these mice had on average ~5% B-1a cells compared to the otherwise normal mice with 22%. The Xid gene expressed on the CBA/N background results in barely detectable numbers of peritoneal B-1a cells, whereas C57BL/6 Xid are reported to have 2–3% B-1a cells (27). Thus, the genetic background can apparently partially determine the penetrance of the Xid gene effects on B-1a cells. Perhaps the higher frequency of cells in the F2 mice we bred reflects a mixed genetic background close to C57BL/6 that allows some maturation of B-1a cells and hence some residual IgA production.

The most interesting questions now concern the function of the IgA produced by B-1 cells of the intestinal tract of the C2d mouse. Kroese et al. have postulated that B-1 cells produce IgA antibody that is important in the relationship of normal intestinal flora to the mucosal immune system (8). If this is so, then one can propose that lack of appropriate IgA that controls intestinal flora or the uptake of antigen released by those flora may contribute to various types of intestinal inflammatory disease. Mice from immune deficiency models, where dysregulation of T cell function has been produced by cytokine gene deletion (IL-2 and IL-10) or transgenic TCRδ expression, develop intestinal inflammation (33–35). SCID mice given CD45RB+ CD4+ T cells also develop colitis (36). All of these are considered CD4 T cell-mediated disease processes and were originally described in mice raised in normal rearing environments (37). When IL-2-deficient mice were made germ free or when SCID mice given CD45RB+ T cells were also treated with antibiotics, both types of intestinal inflammation were prevented (34,38,39). When IL-10-deficient mice were raised in a clean (specific pathogen-free) facility, they had reduced severity and dissemination of colitis, but continue to have focal forms (33). It should be noted here that the C2d mice used in our study do not show any substantial intestinal inflammation or colitis up to 9 months of age, but do develop a severe pancreatitis (manuscript submitted) between 6 and 9 months of age. Thus, we do not observe the colitis first reported among founder strains of C2d mice (35). Our animals are raised in a specific pathogen-free barrier facility and are fully crossed into the C57BL/6 background, which may explain why our C2d mice do not develop colitis. Perhaps C2d mice maintained in a strict specific pathogen-free environment do not develop any such inflammation, because they still maintain B-1 cell function for essential IgA production. We did not examine our F2 mice expressing Xid and lacking MHC class II for occurrence of intestinal inflammation, but the mice appeared healthy until euthanized at 8–10 weeks of age to complete the study.

Our next approach will be to develop a double-deficient mouse (Xid with MHC class II deficiency) on the C57BL6 background. It will be interesting to determine if those mice while harboring normal flora will develop severe intestinal inflammation as they age, when compared to C2d mice. They would then provide an excellent opportunity to investigate the roles of B-1a cell-derived IgA in protection of the intestine from inflammation caused by intestinal bacteria. In addition, they will provide a model for reconstitution of functional IgA production using either normal B-1a cells or (for comparison) normal B-1b cells, in the absence of class II-restricted help by CD4 T cells. This could help resolve the effector roles of B-1a and B-1b cells in IgA production.

Acknowledgements

This work was supported by the MRC Canada and the Ontario Technology Fund.

Abbreviations

AEC 3-amino-9-ethyl-carbazole
C2d class II deficient
CT cholera toxin
MLN mesenteric lymph node

References
B-1a cells and IgA antibody in C2d mice