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Identification and Characterization of a Novel Regulatory Factor: IgA-Inducing Protein

Amy S. Austin,* Karen M. Haas,* Sasha M. Naugler,† Anna A. Bajer,† David Garcia-Tapia,† and D. Mark Estes2*†

IgA is the predominant Ig isotype in mucosal secretions and thus plays a pivotal role in host defense. The mechanisms by which IgA expression is regulated may differ among species and involve multiple pathways. Various cytokines and costimulators have been identified which regulate expression of this isotype, including IL-10, IL-2, vasoactive intestinal peptide, and TGF-β. We have tested a wide array of known factors, but only under very limited conditions do these factors mediate substantial IgA production in vitro from bovine B cells. In response to these findings, we generated a cDNA library in a mammalian expression vector from activated cells derived from bovine gut-associated lymphoid tissues (Peyer’s patch and mesenteric lymph node cells) as a source of soluble factor(s) that may regulate IgA production. We have identified a novel factor, IgA-inducing protein, which stimulates relatively high levels of IgA production in vitro following CD40 stimulation in coculture with IL-2. Our data suggest that IgA-inducing protein regulates IgA by acting as a switch or differentiation factor and is expressed in a variety of lymphoid and nonlymphoid tissues. The Journal of Immunology, 2003, 171: 1336–1342.

One of the primary mechanisms of defense in gut-associated lymphoid tissue (GALT) and secretions is IgA production by B cells. The primary lymphoid organs of the GALT are the Peyer’s patches (PP) and associated areas of the small intestine, but tonsils, adenoids, and appendix are also considered to be part of the GALT (1, 2). IgA is the predominant Ig in normal intestinal mucosa, accounting for 70–90% of all Igss in the GALT (1). IgA provides the first line of defense against many pathogens, functioning in agglutination and neutralization of bacteria, viruses, and toxins. T cells, dendritic cells, and soluble factors regulate type switching to IgA, but the exact mechanisms by which they coordinately control IgA expression are not fully elucidated.

TGF-β is a well-documented switch factor for IgA (3–6). Supporting evidence for the effect of TGF-β on isotype switching and differentiation is provided by TGF-β responsive elements being identified in the regulatory regions of several Cκ genes (7–11). Under defined in vitro culture conditions, TGF-β requires signaling via CD40 and surface IgM to significantly enhance IgA switching and secretion in mice (12). As endogenous TGF-β has dramatic negative effects on both B and T cell proliferation, dual signaling may be necessary to maintain the cell or drive the cell through the cell cycle (13–15).

Several observations suggest that unidentified regulators of IgA in ruminants or other mammalian species may exist, particularly in the environment of the GALT. As TGF-β is ubiquitously found in lymphoid tissues, one could predict that IgA production would be prominent in other sites. However, commitment to IgA expression occurs predominantly in the PP (16). Second, TGF-β at optimal concentrations for cell survival induces only a small fraction of surface IgM+ (sgM+) B cells to undergo IgA switch differentiation. Finally, in cattle, very few regulators of IgA B cell differentiation have been identified. Bovine B cells stimulated via CD40 and anti-IgM in the presence of TGF-β and IL-2 exhibit enhanced production of IgA (17). However, other known IgA regulator factors in rodents and/or humans, including vasoactive intestinal peptide, IL-5, IL-6, and IL-10, have not been evaluated in cattle, but do not induce secretory IgA production from bovine B cells (our unpublished findings). Taken together, these observations suggest that unidentified regulators of IgA+ B cell differentiation, which may work in conjunction with TGF-β, exist in the microenvironment of the gut in cattle.

To identify such factors, we generated a cDNA library in a mammalian expression vector derived from activated cells isolated from the bovine GALT to screen as a source of alternative soluble factor(s) that may regulate IgA production. From this cDNA pool, we have identified a novel factor, IgA-inducing protein (IGIP), which promotes high rate IgA secretion by bovine B cells, with relatively conserved homologues in both humans and mice. We have shown that IGIP induces IgA secretion from B cells stimulated via CD40 alone or a combination of CD40 and BCR signaling. Importantly, IGIP is able to induce IgA production from B cells under stimulation conditions in which TGF-β cannot. Finally, we have shown that IGIP may be expressed in a variety of different tissues, including both lymphoid and nonlymphoid tissues.

Materials and Methods

GALT cDNA library

Isolated lymphocytes from bovine PP and mesenteric lymph nodes collected in a commercial abattoir were stimulated independently with pokeweed mitogen (10 μg/ml; Sigma-Aldrich, St. Louis, MO) or Con A (1 μg/ml; Sigma-Aldrich) or phorbol myristate acetate (10 ng/ml; Sigma-
A plasmid cDNA expression library was constructed from pooled RNA isolated from activated lymphocytes using a commercially available kit (Invitrogen, Carlsbad, CA). The first strand was synthesized using oligo(dT) with a NotI restriction site followed by a Xbal/HindIII adapter ligated into the vector with directional cloning. The cDNA was cloned into an eukaryotic expression vector pcDNA3.1 (Invitrogen). The library was subdivided into 16 random pools.

**Bioactivity expression analysis-GALT cDNA library**

Recombinant protein was produced in COS7 cells (American Type Culture Collection, Bethesda, MD), MOP8 cells (American Type Culture Collection), or BNL-SV A.8 cells (American Type Culture Collection) in serum-free medium (DMEM or ProCHO-4) as indicated. Cells were transfected with lipofectamine (Life Technologies, Gaithersburg, MD) and plasmid DNA according to the manufacturer’s instructions. Seventy-two hours after transfection, cell-free supernatants were collected for bioassay screening as indicated below. Plasmid DNA was reisolated from transfected cells with IgA inductive bioactivity using a commercially available mini-prep kit (Qiagen, Valencia, CA), amplified in Escherichia coli (TOP10F') and subjected to further rounds (two) of enrichment and screening before sequencing was performed on a limited number of clones.

Transfected cell supernatants were analyzed for enhancement of secretory IgA production in culture supernatants following activation of resting B cells with CD40 ligand (CD40L)-transfected L cells and IL-2. Briefly, PBMCs were isolated from 6- to 12-mo-old Holstein or Angus heifers or steers. Red cells were removed by direct lysis (ACK solution; 0.15 M NH₄Cl, 10 mM KHCO₃, 1 mM EDTA). Phagocytic cells were removed by incubation with carbonyl iron and exposure to a magnetic field. B lymphocytes were enriched by panning in the presence of 3% BSA (fraction V; Sigma-Aldrich) in HBSS as previously described (18). Enriched B cells (1 × 10⁶) and 2 × 10⁵ mitomycin C-treated CD40L-DAP3-transfected cells were cultured for 7 days in 96-well plates in 200-μl final volume complete medium (RPMI 1640) supplemented with 10% Ig-free normal horse serum and 1 ng/ml recombinant human IL-2 (PEProTech, Rocky Hill, NJ). Culture supernatant from cells transfected with library plasmids or mock transfec tants were added to 10% of the total culture volume as indicated. In later rounds of screening, further enrichment of resting IgM⁺ B cells was accomplished by negative selection via treatment with a mixture of mouse anti-bovine IgA (Serotec, Raleigh, NC), mouse anti-bovine IgG1 (Serotec), mouse anti-bovine IgG2 (Serotec), and MMIA (anti-CD3 Ab, BD, NJ). Cells positive for these surface molecules were removed using sheep anti-mouse IgG magnetic beads (Dynal Biotech, Oslo, Norway) according to the manufacturer’s instructions. All bioassays were performed in triplicate.

**ELISA**

Assessment of secreted IgM, IgG, and IgA in culture supernatant was assessed by sandwich capture ELISA as previously described (17). Briefly, Immulon II 96-well U-bottom plates (Dynatech, Chantilly, VA) were coated overnight at 4°C with affinity purified goat anti-bovine IgM (μ) (Kirkegaard & Perry Laboratories, Gaithersburg, MD), goat anti-bovine IgG (γ) (Kirkegaard & Perry Laboratories), or goat anti-mouse IgG (γ) (Kirkegaard & Perry Laboratories), respectively. Plates were washed three times with PBS (pH 7.2) and blocked with 10% normal horse serum (Life Technolo gies) in PBS for 1 h at 37°C. Goat anti-mouse IgG-coated plates were incubated for 1 h at 37°C with monoclonal mouse-anti-bovine IgA (Serotec), followed by washing. Bioassay supernatants at various dilutions were added and incubated for 1 h at 37°C. Dilutions of purified bovine IgM (Sigma, St. Louis, MO), IgG (Sigma-Aldrich), or bovine IgA (purified from bovine colostrum using a mouse-anti-bovine IgA column) were assayed in parallel to quantify individual isotypes as appropriate. After washing, plates were incubated with alkaline phosphatase-labeled goat anti-bovine IgM (μ), IgG (γ), or IgG (H + L) (Kirkegaard & Perry Laboratories; for IgA-specific ELISA only) for 1 h. Plates were then washed and incubated with a p-nitrophenylphosphate phosphate substrate kit (Kirkegaard & Perry Laboratories) according to the manufacturer’s instructions. Concentrations of individual Ig isotypes were determined by linear regression relative to known standards. Results are depicted as the mean and SD for triplicate cultures for each condition.

**DNA sequencing**

Clones 2 and 9 were sequenced at the DNA Core Facility at the University of Missouri (Columbia, MO) using the Big Dye Chemistry Sequencing kit (PerkinElmer, Foster City, CA) on an Applied Biosystems 377 DNA Sequenator (PerkinElmer).

**Peptide generation**

Synthetic IGIP peptide (1 μg/μl) was added to 96-well ELISA plates (Immuno II) in PBS overnight at 4°C. Plates were washed three times with PBS-Tween 20 (0.1%) and blocked with Superblock (Pierce, Rockford, IL) according to manufacturer’s instructions. Plates were washed and premixed samples containing test supernatant, known amounts of IGIP peptide, or BSA as a specificity control were preincubated with rabbit anti-IGIP antisera and then added to the ELISA plate. Plates were incubated for 1 h at 37°C and washed. Alkaline phosphatase-conjugated goat anti-rabbit IgG was added to the wells and incubated for 1 h at 37°C. Plates were washed and developed in substrate using a commercial kit (p-nitrophenolphosphate phosphatase; Kirkegaard & Perry Laboratories). Absorbance values were measured at 405 nm. Relative amounts of secreted IGIP in supernatants were determined by linear regression analysis. The correlation coefficient exceeded 0.95.
Bioassay for IGIP activity with highly enriched B cells

Transfected cell supernatants were analyzed for induction of secretory IgA production in coculture with highly enriched B cells (>95% sIgM+ cells) and DAP3-CD40L cells with IL-2. Briefly, PBMCs were isolated from donor calves. Phagocytic cells were removed by incubation with a carbonyl iron suspension followed by gradient isolation (Accuprep, Accurate Chemical, Westbury, NY). Surface IgM-bearing cells were isolated by positive selection using a combination of mouse anti-bovine IgM (BM-23; Sigma-Aldrich) and rat anti-mouse IgG1 microbeads (Miltenyi Biotec). Cells were analyzed postsort by flow cytometry to determine purity. B cells (1% of total cells) were cultured with 2 × 10⁵ mitomycin C-treated CD40L-DAP3-transfected cells for 7 days in RPMI 1640 with 10% normal horse serum. Culture supernatants from cells, transfected with IGIP plasmid (clone 2) or mock-transfected, were added at 20, 10, or 5% of the total culture volume (200 µl) as indicated. Bioassays were performed in triplicate cultures in three independent experiments and the estimations were pooled as the mean and SEM. Estimation of secretory IgA was performed as previously described (20). Statistical significance was determined by Student’s t test following execution and passage of a test for normality. A 95% confidence interval was used to measure significance of IGIP-transfected supernatants vs the mock supernatant at identical concentrations.

Results

Library screening

To identify potential IgA regulatory factors, a cDNA expression library derived from bovine GALT was screened by bioassay using B cells co-cultured with IL-4 and IL-7 in the absence of B cell receptor (BCR) cross-linking. Random plasmid pools were bulk-transfected into BNLSV A.8 cells and the culture supernatant was collected after 72 h. Supernatants from B cell cocultures with transfected supernatants were used to identify cDNAs that enhanced secretory IgA production. Plasmids from each pool that induced the highest IgA production were then further subdivided and reisolated from E. coli and transfected to begin a new round of screening. After three rounds of screening and enrichment, supernatants generated by cDNA transfection of two independent clones (2 and 9) were identified that enhanced relatively high amounts of IgA production by B cells stimulated via CD40 and IL-2 compared to B cells stimulated via CD40 and IL-2 with vector alone (Table I). B cell production of IgM and IgG was also slightly enhanced by supernatants from clone 2 but not by clone 9 in the same assay.

Table I. Secretory IgA in bioassay supernatants following a 7-day coculture of sIgM+ B cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Absorbance at 405 nm</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
</tr>
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<tr>
<td>Vector only</td>
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<td>0.11</td>
<td>0.11</td>
<td>0.18</td>
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<tr>
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<td>0.30</td>
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<td>0.34</td>
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<td>0.11</td>
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<td>Clone 14 plasmid</td>
<td></td>
<td>0.21</td>
<td>0.20</td>
<td>0.17</td>
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</table>

Secretory IgA in bioassay supernatants following a 7-day coculture of sIgM+ B cells with CD40L-transfected cells (B.L. and transfected cell supernatants from clones derived from a GALT expression library. SEM were <10% for all samples.

Expression of IGIP transcripts in tissues and leukocyte subsets

To investigate the potential for IGIP expression, RT-PCR was performed on total RNA extracted from bovine lymphoid and non-lymphoid tissues. IGIP mRNA was present in PP, spleen, thymus, cDNA and predicted amino acid sequence of IgA-enhancing clones

After confirming that BNLSV A.8 transfectant supernatants from cDNA clones 2 and 9 supernatant consistently enhanced IgA production from CD40-stimulated B cells, each cDNA was sequenced and found to be identical (Accession no. AF173827, Fig. 1). Homology searches in GenBank (National Center for Biotechnology Information) suggested a novel cDNA with no significant alignment with genes of known function. The isolated bovine clone 2 cDNA was 96% homologous with a human expressed sequence tag (EST) isolated from a region on chromosome 5 (Fig. 2; Ref. 21). Homology with the EST sequence from human chromosome 5 is of considerable interest, because this chromosome contains genes for several cytokines including IL-3, IL-4, IL-5, IL-13, and GM-CSF (22–27). The nucleotide sequence of clone 2 was 92% homologous with an expressed sequence-tagged (Accession no. AA204132) mouse cDNA clone from mucosal lymph node tissue (Fig. 2). The nucleotide sequence of clone 2 was translated, with the predicted longest complete open reading frame resulting in a peptide of 47 amino acids in length (Fig. 2). This amino acid sequence was 94% homologous with the amino acid sequence of the EST on human chromosome 5 and ~91% homologous with the mouse cDNA isolated from mucosal lymph nodes (Fig. 2). Based on the longest open reading frame within the isolated cDNA clone, the unmodified peptide is predicted to have a molecular mass of ~5.1 kDa and contains a putative signal sequence with a predicted cleavage site between amino acids 23–24 (Expert Protein Analysis System; Swiss Institute of Bioinformatics, Génève, Switzerland). Residues 7, 14, 17, 25, and 33 exhibit variation among the three species compared in Fig. 2. The sequence contains three conserved cysteine residues at positions 9, 28, and 36. A peptide corresponding to amino acids 24–47 with an additional cysteine on the C terminus was synthesized and used to generate a rabbit anti-bovine IGIP Ab as described in Materials and Methods. To confirm the correct open reading frame for the peptide, we used the rabbit anti-bovine IGIP Ab as a direct competitor in our bioassay system. Relative to normal rabbit Ig, rabbit anti-bovine IGIP Ab blocked IgA induction from B cells stimulated with CD40L, IL-2, and IGIP (Table II). This observation suggests that the amino acid sequence used to derive the synthetic peptide is the same amino acid sequence coding for the protein produced by IGIP-transfected cells.

FIGURE 1. Nucleotide and amino acid sequence of clone 2. The top line of each row represents the nucleotide sequence of the library clone (451 nucleotides) and the amino acid sequence of the longest open reading frame (47 amino acids) is shown below the bold-face nucleotides.

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expression of IGIP transcripts in unstimulated and PMA-treated pattern, we sought to clarify the potential for leukocyte subset and at relatively lower levels in the PP. Given the tissue expression appeared similar in spleen, thymus, liver, mesenteric lymph node, and expression levels of IGIP mRNA using semiquantitative methods detect IGIP mRNA in the nasal epithelium (data not shown). Ex-

celar, and mesenteric lymph node and was not detected in the lung, heart, and nasal epithelium (data not shown). Lower panels depict control reactions run with no reverse transcription (RT) or G3PDH as a housekeeping standard for relative loading.

FIGURE 3. Expression analysis of IGIP in various tissues by RT-PCR. IGIP mRNA was present in spleen, thymus, liver, mesenteric lymph node (MLN), and PP but undetectable in normal animals in the lung, heart, and nasal epithelium (data not shown). Lower panels show reverse transcription (RT) or G3PDH as a housekeeping standard for relative loading.

cells. Stimulated or unstimulated peripheral blood-derived CD3+ T cells, CD14+ monocytes, and CD21+ B cells expressed IGIP transcripts at relatively low levels (Table III). Dendritic cells consistently produced the highest levels of steady state IGIP mRNA and expression was increased dramatically by PMA stimulation. To examine the expression of IGIP by dendritic cells under more physiologic conditions, we cocultured CD40L- (CD154) trans-
fected DAP3 cells with peripheral blood-derived dendritic cells and determined the levels of secretory IGIP (Fig. 4) and steady state mRNA (data not shown). IGIP secretion by CD40-activated dendritic cells exceeded that by PMA and ionomycin-stimulated DC by ∼4-fold. These data are consistent with a role for CD154-bearing cells in regulation of IGIP expression by DC. Supernatants from these cells in preliminary studies were also stimulatory (data not shown) on highly enriched B cells (>95% IgM+ B cells) and by recombinant IGIP in transfected cell supernatants. Stimulation of DCs with recombinant IGIP in transfected cell supernatants (Fig. 5) in a dose-responsive manner, suggesting that IGIP has the potential to act on suitably stimulated B cells directly. All comparisons of IGIP-transfected supernatants vs the mock-transfected supernatants at all concentrations used were significantly higher (p < 0.05) by Student’s t test. Mock-transfected supernatants produced relatively lower backgrounds vs previous experiments with CD40 cross-linking alone likely due to the potential contribution of autocrine TGF-β on IgA production under dual ligation conditions with the BCR and CD40. This was necessary to achieve relatively high levels of B cell purification.

Evaluation of the effects of recombinant IGIP and TGF-β on B cells stimulated via CD40

As TGF-β is a key inducer of IgA expression, we sought to examine the effects of IGIP and TGF-β when used alone or in combination following activation of B cells via CD40 (Table IV). B cells stimulated with IGIP supernatant had elevated secretory IgA and expression was increased dramatically by PMA stimulation. To examine the expression of IGIP by dendritic cells under more physiologic conditions, we cocultured CD40L- (CD154) trans-
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indicate that while exogenous TGF-β increases IgA secretion (668 ng/ml). Importantly, our studies showed TGF-β levels in stimulated (PMA) and unstimulated cell subsets. DC, dendritic cells.

Each experiment was repeated independently at least three times from different cell preparations.

FIGURE 4. IGIP is produced by dendritic cells following CD40 cross-linking or via phorbol ester and calcium ionophore treatment. Production of IGIP was analyzed at the transcriptional (data not shown) and translational levels following activation. IGIP production was quantified in supernatants from dendritic cells stimulated in vitro via CD40 or phorbol ester and calcium ionophore. Supernatants were harvested at 24-h poststimulation and analyzed by competitive ELISA. Mean values and SEs are shown for duplicate samples.

FIGURE 5. IGIP induces IgA production by CD40-activated sIgM⁺ bovine B cells. Seven-day culture supernatants were analyzed by capture ELISA following activation with a bovine CD40L-transfected mouse liver fibroblast cell line and IL-2. Results are representative of the mean levels with SEM of IgA production in from three independent pooled experiments using triplicate cultures for each individual experiment (nine data points total) with background levels from comparable mock transfectant culture subtracted (mean ± SEM values for control cultures are as follows: 20% mock-transfected = 771 ± 158; 10% mock-transfected supernatant = 650 ± 88; 5% mock-transfected supernatant = 606 ± 108). Relative amounts of secretory IgA were estimated by linear regression vs a known standard.

|| Cell Type | Unstimulated Cells | Stimulated Cells | Fold Increase |
|---|---|---|---|
| CD3⁺ | 2.36 × 10⁻⁵ | 4.79 × 10⁻⁵ | 2.03 |
| CD14⁺ | 1.55 × 10⁻⁵ | 1.00 × 10⁻⁵ | 0.64 |
| CD21⁺ | 4.79 × 10⁻⁵ | 3.65 × 10⁻⁴ | 7.6 |
| DC | 3.65 × 10⁻⁵ | 0.263 | 7205 |

*M Relative quantification by TaqMan RT-PCR analysis of steady state IGIP mRNA levels in stimulated (PMA) and unstimulated cell subsets. DC, dendritic cells.

** Relative quantification as described in Materials and Methods for duplicate samples.

* Cells were treated with 5 ng/ml PMA for 18 h.

Table IV. Secretory Ig in supernatants following a 7-day coculture of high density sIgM⁺ B cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IgM (ng/ml)</th>
<th>IgG (ng/ml)</th>
<th>IgA (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>Vector only</td>
<td>156 ± 9</td>
<td>&lt;25</td>
<td>303 ± 11</td>
</tr>
<tr>
<td>10% IGIP supernatant</td>
<td>337 ± 115</td>
<td>&lt;25</td>
<td>517 ± 10</td>
</tr>
<tr>
<td>10% IGIP supernatant + TGF-β</td>
<td>160 ± 56</td>
<td>102 ± 24</td>
<td>668 ± 24</td>
</tr>
<tr>
<td>TGF-β</td>
<td>621 ± 232</td>
<td>52 ± 12</td>
<td>245 ± 12</td>
</tr>
</tbody>
</table>

* Secretory Ig in supernatants following a 7-day coculture of high density sIgM⁺ B cells with CD40L-transfected cells, IL-2, and supernatant from clone 2-transfected MOP8 cells vs TGF-β or in combination.

** Mean and SE values for triplicate cultures. Results are representative of three independent experiments.

* 10 ng/ml final concentration.
were T cells using negative selection as described in Materials and Methods. These cells were >70% sIgM+ B cells as determined by flow cytometric analysis.

We and others have shown previously that IL-2 is a key accessory factor for Ig production in general in the bovine (17, 28). IL-2 is not required for IgA expression but enhances the overall amount of IgA secreted by B cells (36, 37). IL-2 has been shown to be essential for IgA expression in multiple species including cattle (38). IL-2 has been proposed to be an important factor in the terminal differentiation of sIgM+ B-2 cells whereas IL-15 has been shown to lead to the enhancement of IgA expression from B-1 but not B-2 cells in mice (39). Mucosal B-1 cell development into IgA-secreting cells has been linked to type 2 cytokines like IL-5. Lack of IL-5R expression in gene-deficient mice results in reduced numbers of B-1 cells and IgA-expressing plasma cells in mucosal tissues (40). Interestingly, recombinant bovine IL-5 has no effect on IgA production by bovine cells activated via the BCR, CD40, or both (D. M. Estes, unpublished findings). The function of IL-5 in later stages of differentiation within schemes of the common mucosal immune system, dependent (B-2) or independent (B-1), awaits further studies in ruminant species (39).

As stimulation of bovine B cells via different receptor molecules can effect the phenotype and effector capabilities of the cell, we examined the effect of IGIP IgA secretion under conditions of dual (CD40 and BCR) or single (CD40) B cell stimulation. Under conditions of dual stimulation, IGIP supernatant induces IgA production by B cells, but exogenous TGF-β augments this effect. Relatively high levels of CD40 engagement in human B cells is sufficient to promote isotype switching to IgA via induction of endogenous TGF-β but not IL-10 (35). In our cell culture model system, exogenous TGF-β did not promote IgA production in the presence of CD40 alone as we have previously reported and consistent with observations in the mouse (12, 17). This finding suggests that CD40 ligation under the conditions used in this study is not in and of itself sufficient to mediate IgA class switching via the production of endogenous TGF-β. CD40 signaling with or without BCR cross-linking promotes the production of IL-10 mRNA by bovine B cells (37). Thus, IL-10 and not TGF-β may promote secretion by cells in transition from sIgM expression to IgA production in the presence of CD40 signaling. Unfortunately, this cannot be directly tested in the absence of a neutralizing Ab reactive with bovine IL-10. It has been proposed that situations in which CD40 signaling occurs at levels sufficient to induce a constant region expression in the absence of exogenous signals allows mucosal B cells to express IgA where T cells may be limiting, such as in contact with dendritic cells expressing functional CD40L. We
are currently pursuing additional studies to clarify the role of IGIP and other factors in regulation of IgA expression in ruminants.

References


