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Monoclonal Immunoglobulin A Derived from Peritoneal B Cells Is Encoded by Both Germ Line and Somatically Mutated V_H Genes and Is Reactive with Commensal Bacteria

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We transferred peritoneal cells from BALB/c mice into C.B17 *scid*/*scid* mice. Six to eight months after injection, only cells with the B1 phenotype were retained in the spleens and peritoneal cavities of these mice. The lamina propria of the intestine contained many peritoneal, donor-derived, immunoglobulin A (IgA)-producing cells. The mesenteric lymph nodes of these mice were found to be a major site of proliferation and generation of IgA plasmablasts. We established eight IgA-producing hybridomas from the mesenteric lymph nodes of such mice, and all the hybridomas reacted with different but partially overlapping fecal bacterial populations. Cloning and sequencing of the V_H genes of these hybridomas showed that two hybridomas utilized germ line-encoded V_H genes while the V_H genes of the other six hybridomas showed somatic mutations, some of which are indicative of an antigen-driven selection process.

Many immunoglobulin A (IgA)-producing plasma cells can be found in the small intestines of conventionally reared mice (44). The specificity repertoire of these IgA-producing cells is largely unknown. Most of this IgA is transported across the epithelium into the gut lumen. The function of this mucosal IgA is still poorly understood. On the one hand, IgA is thought to play an important role in the humoral immune response against pathogenic microorganisms that invade the host at mucosal surfaces (31). For example, administration of monoclonal IgA against such pathogenic microorganisms as *Salmonella typhimurium* and *Vibrio cholerae* results in the rapid disappearance of these pathogens from the intestinal tract (2, 32). On the other hand, many nonpathogenic commensal bacteria have been observed with coatings of IgA antibodies (46). But these coatings do not result in the eradication of these bacteria. In mice kept under germ-free conditions, a very drastic reduction in the total number of IgA-producing cells is observed (43), suggesting an inductive role for the normal microflora in IgA production. The extent and consequences of the interaction between normal gut flora and naturally produced IgA are not clear.

IgA plasma cells in the intestine are generally thought to be derived from precursor B cells that are triggered in Peyer's patches and thereafter migrate to the lamina propria, where they become IgA plasma cells (11). We have shown that significant numbers of IgA plasma cells can be derived from B1 (formerly called Ly-1 B) cells, which reside in the peritoneal cavity (for a review, see reference 26). B1 cells differ from conventional B cells in their phenotype, specificity repertoire, and mode of replenishement (19). They have been shown to be responsible for the production of many multireactive and autoreactive IgM antibodies that are encoded by germ line genes which are not somatically mutated and which express low numbers of N-region insertions (17). Given the notion that many of these IgM antibodies are directed to such epitopes on microorganisms as phosphatidylcholine, dextran, phosphorylcholine (T15 idiotype), and certain determinants on *Escherichia coli* (reviewed in reference 27), we investigated the possibility that B1 cell-derived IgA might be reactive with commensal gut bacteria.

To this end, we transferred peritoneal cells of BALB/c mice into C.B17 *scid*/*scid* mice. Six to eight months after injection, only cells with the B1 phenotype were observed in these mice. Many donor-derived IgA-secreting cells were detected in the intestines of such reconstituted mice. Furthermore, many IgA-positive cells that had recently divided were found in the mesenteric lymph nodes (MLN). We established IgA-producing hybridomas from the MLN cells of such mice and analyzed them for reactivity with the normal gut flora. The V_H genes of the IgA-producing hybridomas were cloned after PCR amplification and analyzed for the presence of N-region insertions and somatic mutations.

MATERIALS AND METHODS

Production of reconstituted mice. BALB/c, C.B17, and C.B17 *scid*/*scid* mice were reared and maintained at our own animal facilities. Eight- to 12-week-old C.B17 *scid*/*scid* mice were injected intraperitoneally with 10^6 or 5×10^6 cells derived from the peritoneal cavities of age-matched, Igh-a allotype BALB/c mice. The animals were analyzed 6 to 8 months after the transfer.

Cell suspensions. Single-cell suspensions from MLN and spleens were prepared by the teasing of tissues in RPMI 1640 and filtration through a Nytex (50-mesh) screen. Intestinal lamina propria cells were collected without density gradient purification as described before (45). Peritoneal cavity (PerC) cells were harvested by injecting 2.5 ml of medium containing serum into the cavity; a small laparotomy was made, and cells were withdrawn with a Pasteur pipette. Three subsequent 2.5-ml washes followed. Typical yields were 4×10^6 to 6×10^6 PerC cells per mouse.

Flow cytometric analysis. Spleen and PerC cells in suspension were stained with monoclonal antibodies (MAbs) specific for total IgM (331.12), IgMa (DS-1), IgMb (78.25), IgDa (AMS 9.1), IgDb (AF6-122), Ly-1 (537.8), and MAC-1 (M1/70) as described previously (26). Anti-IgM antibodies were fluorescein isothiocyanate (FITC)-conjugated, anti-IgD antibodies were biotinylated, and anti-Ly-1 and anti-MAC-1 were allo-phycocyanin conjugated. Conjugated antibodies were a kind gift from A. B. Kantor, Stanford University. Biotinylated antibodies were detected with a streptavidin-phycoerythrin conjugate (Kirkegaard Labora-

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tories Inc.). A total of 30,000 cells were analyzed by three-color flow cytometry with a Coulter Epics Elite cytofluorometer.

ELISA-plaque assays. Enzyme-linked immunosorbent assays (ELISA)-plaque assays of intestinal lamina propria cells were performed essentially as described before (5). Polyvinyl chloride microtiter plates were coated with MAbs specific for total IgA (71.14), IgAa (HY16) (6), and IgAb (HISM2) (26) for the determination of the number of donor- or host-derived IgA plasma cells. Plaques were detected with goat anti-mouse IgA coupled to alkaline phosphatase (Southern Biotechnology Associates, Birmingham, Ala.).

BrdU labelling, cell staining, and fluorescence microscopy. Reconstituted animals were given bromodeoxyuridine (BrdU) (1 mg/ml) in their drinking water for 7 days as described before (12). Drinking water was protected from light during the experiment and was changed every 2 days. MLN cells were stained for IgA with mAb 71-14-FITC and then cytocentrifuged. Slides were fixed in ice-cold ethanol-acetic acid (95:5, vol/vol) for 12 min. To detect BrdU incorporation, the slides were subsequently denatured for 15 min in 2 M HCl at 37°C and incubated with anti-BrdU MAb and then with tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates).

Hybridoma production and ELISA. For hybridoma production, MLN were collected from three C.B17 *scid*/*scid* mice 8 months after the injection of 5×10^6 BALB/c peritoneal cells. MLN cells were pooled and directly fused to Sp2/0 fusion partner cells. Growing hybridomas were screened for immunoglobulin production and, if positive, subsequently for IgM, IgG, and IgA production by ELISA. IgA-producing clones were subcloned in soft agar. The allotype of the produced IgA was determined by ELISA with Hy16 (anti-IgAa) and HISM2 (anti-IgAb) (26) being used as capture antibodies and with goat anti-mouse IgA coupled to alkaline phosphatase (Southern Biotechnology Associates). An ELISA for $\alpha 1 \rightarrow 6$ dextran was done by using 71.14 (anti-IgA) as the capture antibody and $\alpha 1 \rightarrow 6$ dextran-biotin (a kind gift of D. Wang, Columbia University, New York, N.Y.) combined with avidin-alkaline phosphatase conjugate.

Staining of bacteria. Staining of bacteria was done as described in detail elsewhere (46). Bacterial cell suspensions were obtained from fecal samples of C.B17 *scid*/*scid* mice or from human volunteers by centrifugation of 5 g of feces resuspended in 5 ml of phosphate-buffered saline (PBS) for 20 min at $35 \times g$ and 5°C. Samples from the supernatant (containing the bacteria) were taken and incubated with culture fluid from IgA hybridomas. For the screening of monocultured bacterial strains, samples from cultures were suspended in PBS. IgA staining of the bacteria was detected with FITC-conjugated goat anti-mouse IgA (Nordic, Tilburg, The Netherlands). Bacteria were counterstained with 4 μg of propidium iodide per ml. A total of 20,000 bacteria were analyzed on a Coulter Epics Elite cytofluorometer. Bacterial staining was further evaluated by a fluoromorphometrical method developed by the Laboratory of Medical Microbiology, University Hospital Groningen. Use of this method allows quantitative evaluation of the morphology of the stained bacteria. The preparation of slides and fluoromorphometrical analyses were performed as described previously in detail (20). Briefly, 0.5 g of feces derived from C.B17 *scid*/*scid* mice was suspended in 4.5 ml of 0.5% Tween 80 in H_2O and centrifuged at $7 \times g$ for 10 min. The supernatant containing the bacteria was diluted 1:25 in 0.5% Tween 80 solution, and 10 μl was spotted onto a microscope slide. Slides were incubated with 20 μl of the culture fluid of the IgA-producing hybridomas or with culture medium for a negative control. After they were washed, the slides were stained with goat anti-mouse IgA-FITC (Nordic). The slides were evaluated with the image analysis system, and data analysis was performed as described before (20).

Cloning and sequencing of V_H genes. Total cytoplasmic RNA was isolated from about 10^7 hybridoma cells as described before (4). First-strand cDNA reactions were carried out with 200 U of SuperScript II (Life Technologies) in a volume of 20 to 30 μl at 42 to 45°C for 30 to 60 min in the presence of 2 to 5 μg of total RNA, 0.3 mM (each) deoxynucleoside triphosphates (dNTP), 5 to 25 pmol of primer specific for mouse Cα (5′ CTCGGATCCTCACATTCATCGT GCC 3′) (42), 1× first-strand synthesis buffer, and 10 mM dithiothreitol (Life Technologies). The RNA-cDNA hybrid was denatured at 94°C for 5 min, extracted once with phenol-chloroform (1:1) and once with chloroform, and eventually precipitated with ethanol. After centrifugation, the DNA pellet was washed once with 70% ethanol, dried, and dissolved in 20 μl of water. One-tenth of the cDNA obtained this way was amplified in a 50-μl reaction volume containing 35 pmol of VH1BACK primer (5′ AGGTSMARCTGCAGSAGTCWGG ′3 [S = C or G, M = A or C, R = A or G, and W = A or T]) (33), 30 to 35 pmol of primer used for cDNA synthesis (see above), 0.2 mM (each) dNTP, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.05% W-1, and 2.5 to 5 U of *Taq* DNA polymerase (buffers supplied by Life Technologies). PCR cycles consisted of 2 min at 94°C, 2 min at 55°C, and 1.5 min at 72°C for 1 cycle and then 34 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. For hybridomas 1F6, 3C10, 2F7, 1B5, and 2B4, the VH1BACK primer was extended at the 5′ end with an *Hin*dIII site because of internal *Pst*I sites in these V_H genes. PCR products were isolated from a 1.5% agarose gel with a Biotrap (Schleicher & Schuell GmbH, Dassel, Germany), digested with *Pst*I or *Hin*dIII and *Bam*HI, and force cloned into the polycloning site of pUC19. Bacteria (JM83 or DH5α) were transformed by heat shock or electroporation and grown on color selection plates containing an appropriate antibiotic. Color-selected colonies were screened for plasmids containing inserts of the appropriate size and subjected to sequence analyses by dideoxy-mediated sequencing (36). Part of the sequencing reactions were run on an Automated Laser Fluorescent (A.L.F.) DNA Sequencer (Pharmacia Biotech, Roosendaal, The Netherlands). V_H gene sequence alignments were performed with the computer program FASTA (34). Sequences used for alignments were obtained from the EMBL gene bank (12a).

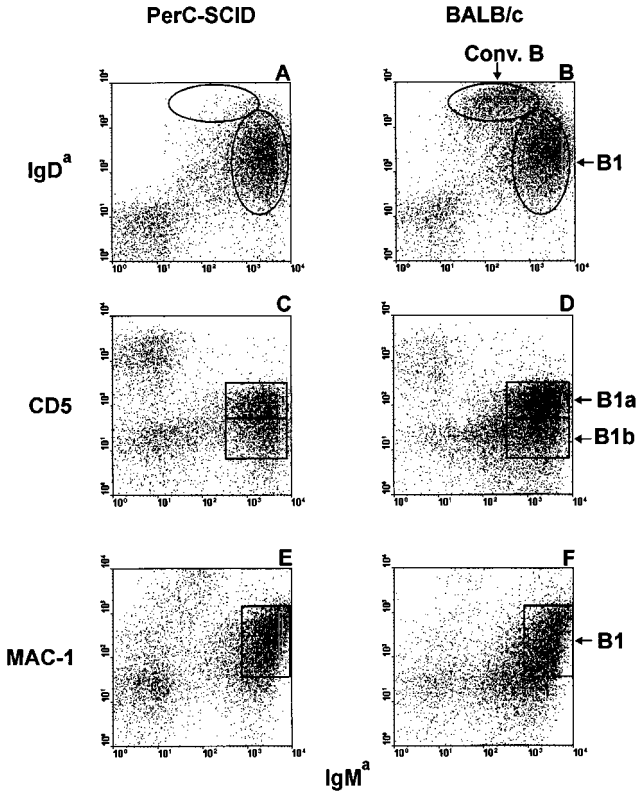

FIG. 1. Flow cytometric analysis of peritoneal cells derived from C.B17 *scid*/*scid* mice (Igh-b) that were injected intraperitoneally with 10^6 peritoneal cells derived from BALB/c mice (Igh-a) 6 months previously (A, C, and E) and from control BALB/c mice (B, D, and F). A total of 20,000 cells were analyzed for IgMa in combination with IgDa (A and B), Ly-1 (CD5) (C and D), and MAC-1 (E and F). Conv. B, conventional B cells.

RESULTS

Flow cytometric analysis. Five C.B17 *scid*/*scid* (Igh-b allotype) mice were injected with 1,000,000 cells harvested from the peritoneal cavities of BALB/c mice (Igh-a allotype). Cytofluorometric analysis of the PerC cells 6 months after injection showed that all PerC donor-derived B cells expressed high levels of IgM (IgM^{high}) and low levels of IgD (IgD^{low}) (Fig. 1). Conventional B cells (IgM^{low}-IgD^{high}) as present in the original donor population were barely detectable. All the PerC B cells in the reconstituted mice expressed MAC-1, as was shown by three-color analysis (Fig. 1E), which is characteristic for PerC B1 cells. Analysis of Ly-1 (CD5) staining (Fig. 1C) showed that about half of the B1 cells expressed CD5. Less than 1% of the PerC cells in these reconstituted mice were stained with b allotype-specific anti-IgM and anti-IgD (data not shown). In the spleens of these mice, less than 7% of the lymphoid cells are B cells (data not shown). In a new set of experiments, 5,000,000 cells derived from the peritoneal cavities of BALB/c mice were injected into three C.B17 *scid*/*scid* mice. Eight months after the injection, reconstituted mice were analyzed for the presence of donor-derived B cells in the spleen and peritoneal cavity. Data were essentially the same as those cited above, showing again that all B cells were IgM^{high} and IgD^{low} in the peritoneal cavity (Fig. 2A). In the second experiment, a large number of B cells were detected in the

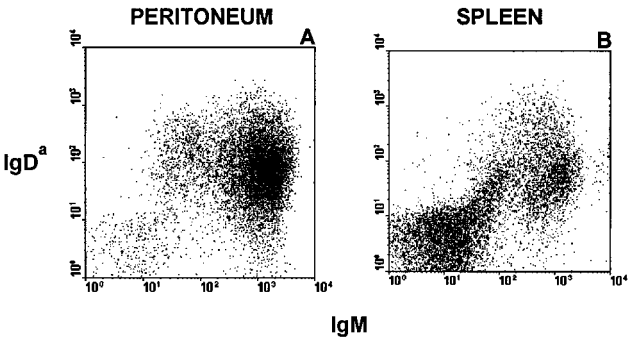

FIG. 2. Flow cytometric analysis of peritoneal (A) and spleen (B) cells derived from C.B17 *scid*/*scid* mice (Igh-b) that were injected intraperitoneally with 5×10^6 peritoneal cells derived from BALB/c mice (Igh-a) 8 months previously. A total of 20,000 cells were analyzed for IgM in combination with IgDa.

spleen, allowing analysis of their phenotype. They were also IgM^{high} and IgD^{low} (Fig. 2B). No appreciable numbers of host-derived B cells were detected, since almost all IgM-positive B cells were stained with a allotype-specific anti-IgD (Fig. 2).

Intestinal IgA-secreting cells. Reconstituted C.B17 *scid*/*scid* mice were analyzed for numbers of total and PerC donor-derived IgA-secreting cells by ELISA-plaque assays of the gut lamina propria 6 and 8 months after the injection of PerC cells from BALB/c mice as described above. As shown by allotype-specific ELISA-plaque assays, virtually all IgA-secreting cells were derived from the PerC cell donor mice (BALB/c) (Table 1). The number of IgA-secreting cells in PerC-reconstituted *scid*/*scid* mice was as high as that in control BALB/c mice.

BrdU labelling, staining of MLN cells, and hybridoma production. In reconstituted mice, the development of Peyer's patches in the small intestine was not macroscopically observed, even though abundant plasma cells were detected in the lamina propria. Staining of fixed cytocentrifuge preparations of MLN cells for cytoplasmic IgA showed that IgA plasma cells constituted 17% of the nucleated cells in these mice. By adding BrdU for 7 days to the drinking water of reconstituted animals at 5 and 8 months after the injection of PerC cells, we could show that most IgA plasma cells had incorporated BrdU (71 to 91%) over a 6- to 7-day period (7). This observation indicated that MLN are a proliferation site for IgA-expressing cells. On the basis of the BrdU experiments, we directly fused MLN cells of three reconstituted mice described above which had been given 5×10^6 PerC cells 8 months previously. From a total of 174 wells that were plated out, we obtained 76 growing hybridomas. Forty-six of the 76 growing hybridomas produced immunoglobulin and eighteen of them produced IgA (39% of the immunoglobulin-producing clones), as determined by ELISA. Subcloning of the IgA hybridomas resulted in eight stable IgA-producing clones that have been further analyzed. By allotype-specific ELISA, we could show that all eight IgA-producing hybridomas were derived from the BALB/c PerC donor (data not shown).

TABLE 1. Numbers of IgA-secreting cells in the small intestines of C.B17 *scid*/*scid* mice after injection of BALB/c mouse-derived peritoneal cells

Donor cell (no.)	Mo after transfer	Mean no. ± SEM (10^6)[a]		
		IgAa	IgAb	IgA total
PerC BALB/c (10^6)	6	40 ± 24	0.5 ± 0.3	18 ± 6
PerC BALB/c (5×10^6)	8	49 ± 6	ND	25 ± 1
Control BALB/c		46 ± 19	0	34 ± 22
Control C.B17		0	16 ± 4	29 ± 9

[a] Numbers were determined in ELISA-plaque assays with three to five individually tested mice by coating with MAbs Hy16 (anti-IgAa), HISM2 (anti-IgAb), and 71.14 (anti-IgA total). Development of the plaques was done with goat anti-mouse IgA coupled to alkaline phosphatase. For purposes of comparison, the numbers from control BALB/c ($n = 5$) and C.B17 ($n = 5$) cells are shown. ND, not done.

TABLE 2. Staining of bacteria derived from human and C.B17 *scid*/*scid* mouse fecal samples by IgA MAbs as analyzed by flow cytometry[a]

Fecal sample (no.)	Mean % ± SEM[b]							
	1B5	2F7	2D11	1F6	3G8	3C10	3C6	2B4
Human (2)	7 ± 2	11 ± 3	4 ± 1	2 ± 1	9 ± 2	4 ± 0.3	5 ± 2	15 ± 5
C.B17 *scid*/*scid* mouse (3)	13 ± 2	6 ± 1	7 ± 0.1	6 ± 1	4 ± 1	3 ± 1	4 ± 2	5 ± 2

[a] Bacteria were stained by incubating them with the culture fluids of the indicated IgA-producing hybridomas and then washing them with goat anti-mouse IgA-FITC.

[b] Percentages were calculated by subtracting the percentage of negative control (culture fluid) staining from that of IgA staining with the Immuno-4 program (Coulter).

Flow cytometric analysis of bacterial staining. Since IgA is primarily involved in immune responses at mucosal sites and some IgM-producing B1 cells are known to have antibacterial specificities, we analyzed the reactivity of our IgA MAbs with bacteria derived from the intestinal tract. Since it can be expected that a large percentage of the intestinal bacteria are coated with the IgA that is being produced by the mouse itself, we used sources of intestinal bacteria that were free of coating with mouse-derived IgA to test the reactivity of our IgA MAbs. For this purpose, fecal bacteria from human volunteers and from *scid*/*scid* mice were used. Flow cytometric analysis of both bacterial samples incubated with supernatant of the IgA hybridomas revealed that all eight IgA MAbs stained different percentages (2 to 15%) of the bacteria (Table 2). The percentages of bacteria that were stained with each IgA sample were similar in three independent experiments, indicating that the distribution of bacteria in the fecal samples used was rather invariable. For the *scid*/*scid* mice, we used a pool of fecal samples from a group of 15 mice, while the human samples originated from one donor. The percentages of human- and mouse-derived fecal bacteria that were recognized by a certain MAb were remarkably alike, suggesting comparable compositions of the recognized bacteria of human and mouse fecal flora. The different percentages that were stained with each of the IgA MAbs indicate that these MAbs recognized, at least in part, different bacteria or bacterial epitopes. This suggestion was confirmed by combining different MAbs in one staining, which resulted in partially additive percentages of stained bacteria (data not shown).

Flow cytometric analysis with the different IgA MAbs of monocultures of strains of facultative anaerobic bacteria of the taxa *Enterococcus*, *Staphylococcus*, and *Enterobacteriaceae* isolated from fecal samples of C.B17 *scid*/*scid* mice showed that these MAbs did not bind these bacteria (data not shown). For three hybridomas, reactivity with monocultures of strictly anaerobic strains could be shown (Fig. 3). 2B4 reacted with *Peptostreptococcus productus*, 2F7 reacted with *Peptostreptococcus anaerobius*, and 1B5 showed binding to *Clostridium perfringens.*

Morphometric analysis of bacterial staining. To obtain more information on what kind of bacteria were stained with

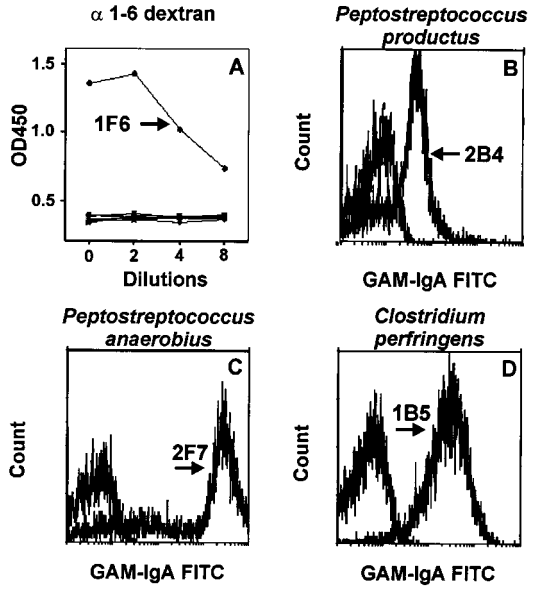

FIG. 3. Reactivity of IgA hybridomas to bacterial antigens. (A) Supernatants of IgA hybridomas were tested by α1→6 dextran-specific ELISA, and only 1F6 showed binding to α1→6 dextran compared with a negative control (culture medium) and the other seven hybridomas. OD450, optical density at 450 nm. (B) Flow cytometric histograms of staining of the indicated monocultured bacterial strains with the indicated IgA MAbs and of control staining (with culture medium) followed by staining with goat anti-mouse (GAM) IgA-FITC (B, C, and D).

our IgA MAbs, we used a recently developed immunomorphometrical method (47). By this method, bacterial samples are screened for different morphological parameters, such as size and shape, resulting in two-dimensional diversity patterns for the bacterial forms which describe most (i.e., 93.5%) of the original variance in combination with immunofluorescence staining. The fluorescence distribution patterns of the different observed bacterial forms obtained from *scid*/*scid* mouse fecal samples for the eight IgA MAbs in combination with the patterns of the observed forms are shown as contour plots in Fig. 4. Different distribution patterns of the staining of the bacterial forms were observed with our IgA MAbs. Although the morphological distribution of bacteria (morphotypes) does not directly correlate with bacterial species, the distribution pattern of the staining of these morphotypes can be indicative for the groups of bacteria being recognized (47). For instance, IgA MAb 2D11 preferentially stained more small rod-like bacteria, while staining with MAb 2F7 is more confined to the larger coccoid forms. Furthermore, the similarities in staining intensities by the different MAbs can be quantitatively analyzed by this method. The F1-F2 plane was divided into 12-by-12 fields, or morphotypes, and a fluorescence intensity for each of the 144 morphotypes was determined. A similarity coefficient was calculated by comparing the observed fluorescence intensities of different IgA MAb samples, which resulted in a value between 0 and 1, with 0 meaning no overlap and 1 meaning 100% overlap in the distribution of the fluorescence signal and the morphotypes. A significance criterion of 0.465 was set as the mean of two similarity coefficients of stainings with one IgA MAb on the same fecal sample minus twice the standard deviation. Table 3 shows the similarity coefficients for the staining of the bacteria with the different MAbs. The results show that MAbs 2B4, 3C6, 2F7, and 2D11 have staining patterns which are significantly different from other staining patterns (Fig. 4). On the other hand, the overlap in staining with MAbs 1F6, 3G8, 1B5, and 3C10 is such that no significant differences can be seen in their distribution patterns by this method (Fig. 4).

In a result similar to that of our flow cytometric analysis, immunomorphometric analysis of purified samples of strains of the genera *Enterococcus*, *Staphylococcus*, *Enterobacter*, and *Pseudomonas* isolated from *scid*/*scid* mice did not show any significant staining with the IgA MAbs of these bacteria.

V_H gene usage of the IgA-producing hybridomas. We cloned the V_H genes from the hybridomas by RNA PCR. The sequences are shown along with sequences obtained from the EMBL data bank of the most homologous known germ line V_H genes (Fig. 5). Table 4 shows the origins (hybridoma or germ line name) of those sequences and the known specificities of the hybridomas.

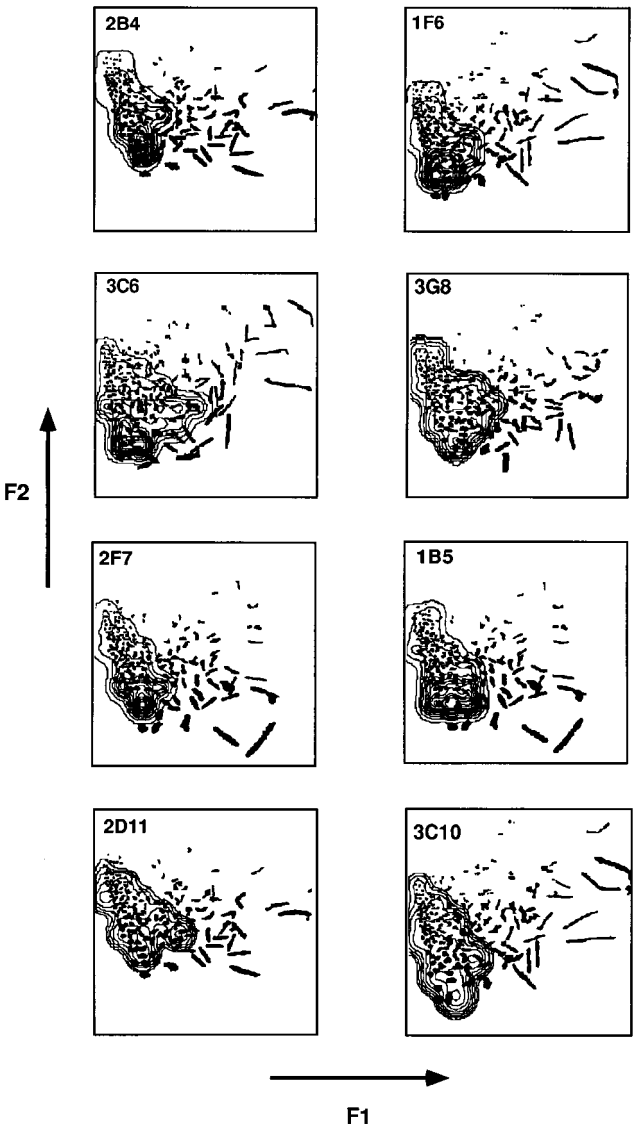

FIG. 4. Contour plots of bacteria from fecal samples derived from C.B17 *scid*/*scid* mice that were analyzed morphometrically in combination with immunofluorescent staining with the indicated IgA MAbs. Bacterial forms were described by factor 1 (F1) and factor 2 (F2), which describe 93.5% of the original variance. In the graphs, representatives of the observed bacterial forms are drawn. The distribution of staining by the indicated IgA MAb followed by staining with goat anti-mouse IgA-FITC is represented as contour plots.

TABLE 3. Similarity coefficients of the spread of the staining by IgA MAbs over the different forms of bacteria

Hybridoma	Similarity coefficient[a]							
	2F7	1B5	3G8	2B4	2D11	1F6	3C10	3C6
2F7	1.000							
1B5	0.592	1.000						
3G8	0.593	0.955	1.000					
2B4	0.481	*0.306*	*0.373*	1.000				
2D11	*0.372*	0.704	0.594	0.198	1.000			
1F6	0.652	0.931	0.905	0.442	0.638	1.000		
3C10	0.631	0.881	0.915	*0.379*	0.624	0.850	1.000	
3C6	*0.004*	*0.023*	*0.014*	*0.328*	*0.257*	*0.002*	*0.102*	1.000

[a] Numbers represent the similarity coefficients of the two compared stainings as calculated by the GRID system. Numbers in italic type represent significantly different staining patterns. The significance criterion of 0.465 was set as the mean of two similarity coefficients of stainings with one IgA MAb on the same fecal sample minus twice the standard deviation.

The V_H gene of hybridoma 1F6 differs by only two nucleotides from a germ line V_H gene which has been shown to be used in an IgA-producing hybridoma reactive with $\alpha1\rightarrow6$ dextran (38). Remarkably, 1F6 had, next to a virtually identical germ line V_H gene, a V_H-D border identical to that of $\alpha1\rightarrow6$ dextran-specific hybridoma 26.4.1 and only three differences at the D-J_H3 border, and utilization of DFL16.3 was the same for the two hybridomas (Fig. 6 shows the CDR3 sequence). We confirmed the $\alpha1\rightarrow6$ dextran specificity of 1F6 by ELISA (Fig. 3).

Also, hybridoma 2D11 utilizes a V_H gene which has a high percentage identity, although with a large number of differences, with another germ line V_H gene known to be used for $\alpha1\rightarrow6$ dextran specificity (1). 2D11 showed no $\alpha1\rightarrow6$ dextran specificity by ELISA (Fig. 3). Hybridoma 3C6 utilizes a V_H gene which has considerable homology with a germ line V_H gene which was originally isolated from an autoreactive IgM-producing hybridoma of a motheaten mouse (23). The V_H gene of hybridoma 3C10 is best compared with the V_H gene originally isolated from an autoreactive IgM-producing hybridoma derived from an MLR/lpr mouse (41). Two hybridomas (1B5 and 2B4) utilize PC7183 germ line genes for which the specificities have not yet been described (10). Finally, 2F7 and 3G8 utilize V_H genes that have been described as being used in hybridomas with specificities for such substances as oxazolon and 2,4-dinitrophenol (21, 28) (Table 4). Testing of our IgA hybridomas for oxazolon and 2,4-dinitrophenol specificity by ELISA showed no reactivity with those antigens (data not shown).

Occurrence of somatic mutations in the V_H genes. V_H genes in the data bank are considered to be germ line, either because they were isolated as such or because multiple identical (>99%) samples of their sequences were present in the EMBL data bank (15). All progenitor V_H genes used for purposes of comparison have been proven to be of germ line origin, except for AN07. However, this V_H gene most likely closely resembles a germ line V_H gene, since it has only three mismatches with another independently isolated V_H gene. Two of our IgA hybridomas (2F7 and 1F6) were identified as utilizing germ line V_H genes. For the other six hybridomas, we found 7- to 32-nucleotide differences with the best-fitting germ line V_H gene. If nucleotide differences can be identified when V_H genes are compared with the closest related known germ line gene, the nature of these differences can be indicative of random or selected mutations. Usually, antigen-selected V_H genes exhibit relatively more somatic mutations in the complementary determining regions (CDR) regions than in the framework regions (FR) regions. In addition, a higher replacement-to-silent-mutation (R/S) ratio is observed in the CDR (3). However, the codon usage in the FR and CDR also influences the chances of replacement or silent mutations (8). The expected R/S ratio was calculated on the basis of the codon usage of the progenitor germ line V_H gene, and the chances that the found excess of replacement mutations in our V_H sequences occurred at random were estimated (Table 4).

By comparing the observed and the expected excesses of replacement mutations in the FR and CDR, we showed for five of our IgA hybridomas that the chances that these differences occurred at random were less than 5% (Table 4). However, the differences between the V_H genes of hybridoma 2D11 and hybridoma 19.1.2 span 5 nucleotides on a row in FR3 (Fig. 5), which is very unlikely to have occurred by somatic mutation. More likely, this V_H gene is not the right progenitor germ line for 2D11. For hybridomas 2B4 and 3C6, the differences in the CDR were not significantly higher than expected from the R/S ratios, but the differences in the FR were significantly lower than expected. Hybridomas 3G8 and 3C10 exhibited higher R/S ratios in the CDR then expected, suggesting that at least these two IgA MAbs originated from antigen-selected B cells.

CDR3 length, D regions, and number of N insertions. By comparing the sequences of the CDR3 with those of known D and J_H regions, we could identify the different D and J_H regions that are used in the IgA hybridomas and the number of N insertions between these regions (Fig. 6). The usage of the D region is very variable (Fig. 6). In hybridomas 2F7 and 2B4, it was very difficult to assign the D region to one known germ line D region. 2F7 has a D region which partly resembles DFL16 and partly the DQ52 gene, while 2B4 has a mixture of DQ52 and DSp2.5. Six of the hybridomas use J_H3, while J_H2 and J_H4 are both used once. On the basis of N insertions at both the V-D and the D-J_H junctions, the hybridomas can be divided into two groups. One group (2F7, 1F6, 3C6, 3C10, and 1B5) has no or few ($\leq$2) N insertions, while the other group (2D11, 2B4, and 3G8) has more than five N insertions, especially at the V_H-D border. The two hybridomas that utilize germ line V_H genes also show very few N insertions. 2F7 has only 1 extra nucleotide between the V_H and the D regions and none at the 3′ border. 1F6 has only one extra C at both ends of the D regions. The length of the CDR3, which varies between 12 and 36 nucleotides, was very variable among germ line V_H genes. For example, 2F7 has a CDR3 of only 17 bp, while that of 1F6 is 30 bp long.

DISCUSSION

Our previous experiments demonstrated that intestinal IgA plasma cells can originate from B1 cells (25). Here, we have confirmed and extended these observations by transferring peritoneal cells into *scid*/*scid* mice. In adult mice, B1 cells have a self-renewal capacity, in contrast to conventional B cells, which are replenished by bone marrow precursors (19). Therefore, after transfer of peritoneal cells into *scid*/*scid* mice, B1 cells will be able to maintain in the host over a long time. Without bone marrow precursors, the conventional B cells are not able to maintain themselves by self-replenishment and die at several months after transfer. In such reconstituted mice, high numbers (40,000,000) of IgA-secreting cells are found in the gut lamina propria at more than 6 months after transfer.

We have several reasons to believe that these IgA-secreting cells are predominantly (or all) derived from the B1 cell population. First, only cells with the B1 cell phenotype can be detected both in the peritoneum and in the spleen in such mice at more than 6 months after reconstitution. However, other

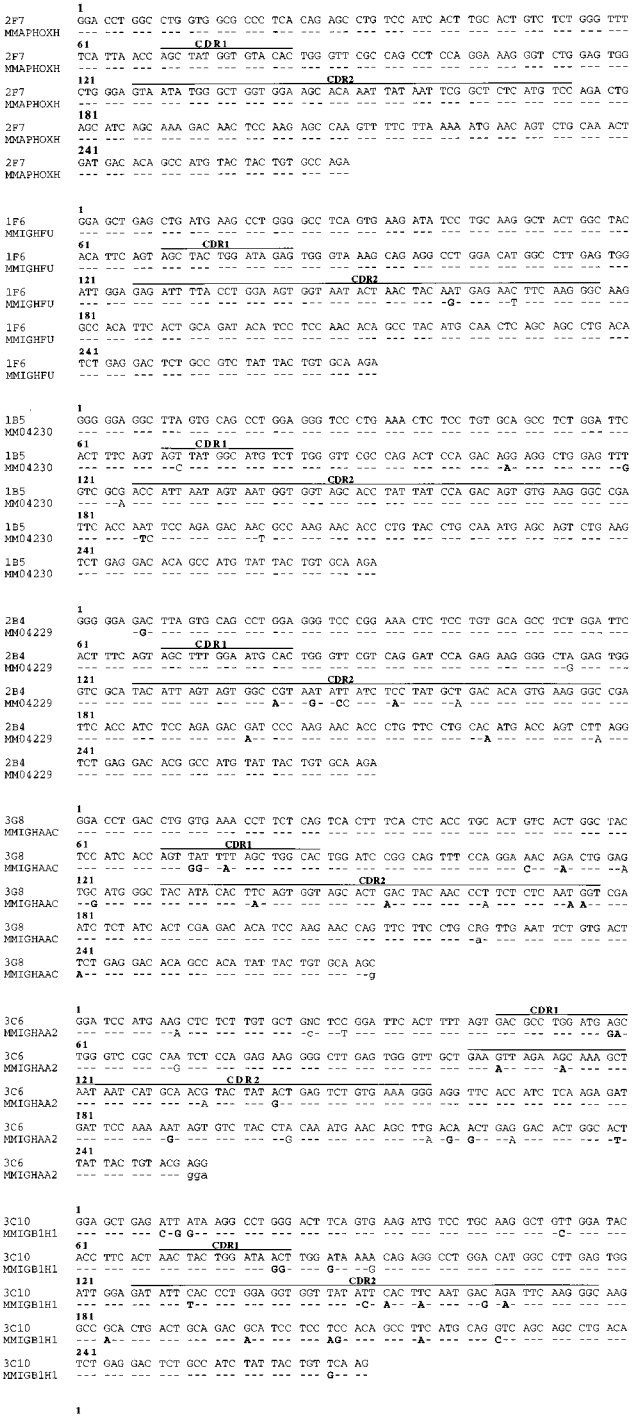

FIG. 5. Nucleotide sequences of the V_H genes from the IgA hybridomas. Sequences start at codon 8, according to Kabat et al. (21a), except for that of hybridoma 3C6, which starts at codon 15 because of an internal *Bam*HI site. The first two nucleotides are derived from the 3′ end of the universal V_H primer. For purposes of comparison, the germ line V_H gene with the highest homology is shown. Identical nucleotides are indicated by hyphens, replacement mutations are shown in boldface, and silent mutations are shown in lightface. Lowercase letters indicate what are most likely junctional differences or sequence ambiguities that are not considered somatic mutations in Table 4. The borders of CDR1 and CDR2 are indicated.

investigators could show recovery of B cells (with uncertain B1 or conventional B-cell phenotypes) up to 10 months after the transfer of large numbers (10,000,000 to 50,000,000) of lymph node cells (containing mostly conventional B cells) into *scid*/*scid* mice (40). Since we injected only small numbers of conventional B cells (from 0.5×10^6 to 2×10^6) and conventional B cells slowly decline without self-renewal (22), we assume that we waited long enough after transfer for the extinction of the injected conventional B cells. Second, in the MLN of these reconstituted mice, a large proportion of the IgA-positive cells had recently divided. This finding, in combination with the observed short half-life of IgA plasma cells (30), makes it very likely that these IgA-positive cells are derived from B1 cells. Third, V_H gene usage of the IgA-producing hybridomas produced from these MLN cells provides supportive evidence for their B1 cell origin. Hybridoma 1F6 has, next to a virtually identical germ line V_H gene, a V_H-D border identical to that of α1→6 dextran-specific hybridoma 26.4.1 and only three differences at the D-J_H3 border (38), and utilization of DFL16.3 was the same for the two hybridomas, which was confirmed by the α1→6 dextran specificity of 1F6 by ELISA. Anti-dextran antibodies are mostly produced by B1 cells (13). Furthermore, hybridoma 3C6 utilizes a V_H gene which has high homology with a germ line V_H gene which was originally isolated from an autoreactive IgM-producing hybridoma of a motheaten mouse, which has only B1 cells (23). The V_H gene of hybridoma 3C10 has high homology with the V_H gene originally isolated from an autoreactive IgM-producing hybridoma derived from an MRL/lpr mouse (41). Fourth, B1 cell-derived IgM usually lacks somatic mutations and shows low numbers of N insertions in the used V_H genes (14). The V_H genes of hybridomas 2F7 and 1F6 seem to fulfill both these criteria as IgA-producing cells, providing indirect evidence for the B1 cell origin of these hybridomas. Furthermore, we found fewer than two N-region insertions at the V_H-J border and the D-J_H border in five hybridomas, which is low compared with the published number of N-region insertions in conventional B cells (14). This finding provides supportive evidence for the B1 origin of these IgA hybridomas.

As a result of V_H gene sequence analysis of six IgA-producing hybridomas, we found a number of nucleotide differences compared with the sequence of the best-fitting V_H gene. However, only hybridomas 3G8 and 3C6 show a pattern of somatic mutations that is indicative of possible antigen selection. In humans, somatic mutations have been shown in CD5-positive B cells (29), while such mutations have not been shown for mouse B1 cells. This finding might be explained by the fact that until now, only genes encoding B1 cell-derived IgM have been sequenced (18). Alternatively, 3G8 and 3C6 might be derived from conventional B cells.

Finally, all of our IgA MAbs react with the bacterial epitopes of the normal gut flora. This finding is in accordance with the observed specificities of B1 cell-derived IgM antibodies, which have been shown to be directed to epitopes on microorganisms (reviewed in reference 27). Furthermore, B1 cells have been shown to play a role in protection against *Salmonella* infections (35).

The reactivity of IgA with the commensal flora is largely unknown. In humans, many of the nonpathogenic commensal bacteria are coated with IgA antibodies (46). Furthermore,

TABLE 4. Occurrence of somatic mutations in the V_H regions of IgA-producing hybridomas

Hybridoma	Origin of compared V_H gene	Family of compared V_H gene	GL[a]	Specificity of compared V_H gene	Reference	% Identity	No. of mutations	Observed R/S ratio for FR	Expected R/S ratio for FR[b]	*P* value for FR[c]	Observed R/S ratio for CDR	Expected R/S ratio for CDR[b]	*P* value for CDR[c]
2F7	H26	Q52	+	Oxazolon	21	100.0	0						
1F6	26.4.1	J558	+	α1→6 dextran	38	99.3	2	∞ (0/0)	3.1643	0.181	1.0 (1/1)	4.4412	0.319
1B5	68-5N	PC7183	+	?	10	97.4	7	1.0 (3/3)	3.0276	0.223	0.0 (0/1)	3.7750	0.222
2B4	61-1P	PC7183	+	?	10	95.9	11	1.5 (3/2)	2.9345	0.032[d]	2.0 (4/2)	4.1351	0.087
3G8	AN07	36-60		2,4-Dinitrophenol	28	94.9	13	4.0 (4/1)	2.9139	0.041[d]	7.0 (7/1)	4.5000	0.005[d]
3C6	UN 42.5	J606	+	TC and RBC[e]	23	93.2	16	0.7 (4/6)	3.4522	0.011[d]	5.0 (5/1)	4.1250	0.159
3C10	7B6.8	J558	+	Kidney	41	92.1	21	12.0 (12/1)	2.9931	0.173	0.0 (8/0)	3.9730	0.027[d]
2D11	19.1.2	J558	+	α1→6 dextran	1	88.5	32	1.1 (11/10)	3.1643	0.005[d]	1.8 (7/4)	4.4118	0.162

[a] GL, known germ line origin of compared V_H gene. All V_H genes used for comparison are germ line genes, except for AN07, which has three mismatches with another independently isolated V_H gene.
[b] The expected R/S ratios were calculated on the basis of codon usage in the FR and CDR regions as described previously (33).
[c] The *P* values represent the chances that the observed mutations occurred without selection.
[d] Significant differences between observed and expected R/S ratios ($P < 0.05$).
[e] TC, thymocytes; RBC, erythrocytes.

many human hybridomas derived from the MLN of patients with inflammatory bowel disease react with bacteria of the normal gut flora (9).

Morphometrical analysis and additive staining with combinations of our MAbs showed that the IgA MAbs recognize partially overlapping and partially unique bacterial populations. The overlapping recognition can be caused either by interaction of the conserved regions in the antibodies, such as the FR or the IgA constant regions, or by binding to oligosaccharides of the IgA glycoprotein. For instance, human IgA has been shown to bind to the mannose-specific lectin of *E. coli* (48). Alternatively, binding to B-cell superantigens might give an explanation of this overlapping staining pattern. *Staphylococcus aureus*-derived protein A has been shown to act as a B-cell superantigen by reacting with the FR3 regions of the heavy chain coded for by certain groups of both mouse (S107 and J606) and human V_H (group III) genes (37, 39). One of our IgA MAbs (3C6) did react with protein A (data not shown), which correlated well with the usage of the J606 V_H gene family. In this respect, it is remarkable that in four of our hybridomas, many replacement mutations were found in FR3, suggesting clonal selection on FR3-binding determinants.

The reactivity patterns of our IgA MAbs with commensal gut bacteria suggest a role for IgA in establishing the homeostasis between the host and the normal gut flora. Whether B1 cell-derived IgA differs functionally from conventional B cell-derived IgA is currently unknown. Much of the IgA that is produced in neonatal mice is reactive with the commensal bacteria that are then seen for the first time (24) in a situation in which the immune system is dominated by B1 cells (16).

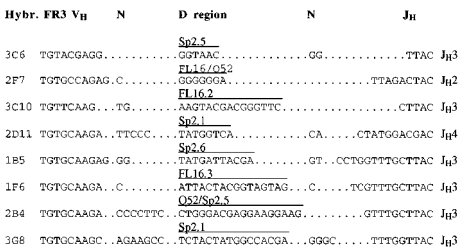

FIG. 6. Nucleotide sequences of the CDR3 of the IgA hybridomas. For purposes of orientation, the last three codons of FR3 are also shown. The CDR3 are divided into N insertions, D regions, and J_H regions. The D regions of hybridomas 2F7 and 2B4 partially match FL16-Q52 and Q52-and Sp2.5, respectively. Hybr., hybridoma.

Therefore, we speculate that B1 cell-derived IgA might provide the animal with a first line of humoral defense to most commonly encountered antigens. This activity results in a balanced situation in which there is a homeostasis between IgA production in the host by self-renewing B1 cells and maintenance of a normal gut flora. Later encounters with (pathogenic) bacteria which disturb this homeostasis result in humoral immune responses through Peyer's patches, and this immune response is dominated by conventional B cells.

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REFERENCES

1. **Akolkar, P. N., S. K. Sidker, S. B. Bhattacharya, J. Liao, F. Gruezo, S. L. Morrison, and E. A. Kabat.** 1987. Different VL and VH germ-line genes are used to produce similar combining sites with specificity for α(1-6)dextrans. J. Immunol. **138:**4472–4479.
2. **Apter, F. M., P. Michetti, L. S. Winner III, J. A. Mack, J. J. Mekalanos, and M. R. Neutra.** 1993. Analysis of the roles of antilipopolysaccharide and anti-cholera toxin immunoglobulin A (IgA) antibodies in protection against *Vibrio cholerae* and cholera toxin by use of monoclonal IgA antibodies in vivo. Infect. Immun. **61:**5279–5285.
3. **Berek, C., and M. Ziegner.** 1993. Somatic hypermutation and affinity maturation. Immunol. Today **14:**400–404.
4. **Bos, N. A., and C. G. Meeuwsen.** 1989. B cell repertoire in adult antigen-free and conventional neonatal BALB/c mice. I. Preferential utilization of the C_H-proximal V_H gene family PC7183. Eur. J. Immunol. **19:**1811–1815.
5. **Bos, N. A., C. G. Meeuwsen, B. S. Wostmann, J. R. Pleasants, and R. Benner.** 1988. The influence of exogenous antigenic stimulation on the specificity repertoire of background immunoglobulin-secreting cells of different isotypes. Cell. Immunol. **112:**371–380.
6. **Butcher, E. C., R. V. Rouse, R. L. Coffman, C. N. Nottenburg, R. R. Hardy, and I. L. Weissman.** 1982. Surface phenotype of Peyer's patch germinal center cells: implications for the role of germinal centers in B cell differentiation. J. Immunol. **129:**2698–2707.
7. **Cebra, J. J., N. A. Bos, E. R. Cebra, G. J. Deenen, and F. G. M. Kroese.** B1 cells are selectively retained in the peritoneal cavity and both B1 cells and IgA+ B2 cells can contribute to the pool of murine IgA plasmablasts over long periods. Submitted for publication.
8. **Chang, B., and P. Casali.** 1994. The CDR1 sequences of a major proportion of human germline Ig V_H genes are inherently susceptible to amino acid replacement. Immunol. Today **15:**367–372.
9. **Chao, L. P., J. Steele, C. Rodrigues, J. Lennard-Jones, J. L. Stanford, C. Spiliadis, and G. A. W. Rook.** 1988. Specificity of antibodies secreted by hybridomas generated from activated B cells in the mesenteric lymph nodes of patients with inflammatory bowel disease. Gut **29:**35–40.
10. **Chukwuocha, R. U., A. B. Hartman, and A. J. Feeney.** 1994. Sequences of four new members of the V_H7183 gene family in BALB/c mice. Immunogenetics **40:**76–78.

11. **Craig, S. W., and J. J. Cebra.** 1975. Rabbit Peyer's patches and popliteal lymph node B lymphocytes: a comparative analysis of their membrane immunoglobulin components and plasma cell precursor potential. J. Immunol. **114:**492–502.
12. **Deenen, G. J., and F. G. M. Kroese.** 1993. Kinetics of B cell subpopulations in peripheral lymphoid tissues: evidence for the presence of phenotypically distinct short-lived and long-lived B cell subsets. Int. Immunol. **5:**735–741.

12a. **European Molecular Biology Laboratory.** 1994. Data library release 41. European Molecular Biology Laboratory, Heidelberg, Germany.

13. **Förster, I., and K. Rajewsky.** 1987. Expansion and functional activity of Ly-1+ cells upon transfer of peritoneal cells into allotype-congenic newborn mice. Eur. J. Immunol. **17:**521–528.
14. **Gu, H., I. Förster, and K. Rajewsky.** 1990. Sequence homologies, N sequence insertion and J_H gene utilization in V_HDJ_H joining: implications for the joining mechanism and the ontogenetic timing of Ly-1 B cell and B-CLL progenitor generation. EMBO J. **9:**2133–2140.
15. **Gu, H., D. Tarlington, W. Müller, K. Rajewsky, and I. Förster.** 1991. Most peripheral B cells in mice are ligand selected. J. Exp. Med. **173:**1357–1371.
16. **Hardy, R. R., and K. Hayakawa.** 1986. Development and physiology of Ly-1 B cells and its human homolog, Leu-1 B. Immunol. Rev. **93:**53–79.
17. **Hardy, R. R., and K. Hayakawa.** 1992. Developmental origins, specificities and immunoglobulin gene biases of murine Ly-1 B cells. Int. Rev. Immunol. **8:**189–207.
18. **Hayakawa, K., C. E. Carmack, R. Hyman, and R. R. Hardy.** 1990. Natural autoantibodies to thymocytes: origin, Vh genes, fine specificities, and the role of Thy-1 glycoprotein. J. Exp. Med. **172:**869–878.
19. **Herzenberg, L. A., A. M. Stall, P. A. Lalor, C. Sidman, W. A. Moore, D. R. Parks, and L. A. Herzenberg.** 1986. The Ly-1 B cell lineage. Immunol. Rev. **93:**81–102.
20. **Jansen, G. J., M. H. F. Wilkinson, B. Deddens, and D. Van der Waaij.** 1993. Characterization of human faecal flora by means of an improved fluoromorphometrical method. Epidemiol. Infect. **111:**265–272.
21. **Kaartinen, M., M.-L. Solin, and O. Mäkelä.** 1989. 'Allelic' forms of immunoglobulin V genes in different strains of mice. EMBO J. **8:**1743–1748.

21a. **Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman, and C. Foeller.** 1991. Sequences of proteins of immunological interest, 5th ed. NIH publication no. 91-3242. National Institutes of Health, Bethesda, Md.

22. **Kantor, A. B., A. M. Stall, S. Adams, K. Watanabe, and L. A. Herzenberg.** 1995. De novo development and self-replenishment of B cells. Int. Immunol. **7:**55–68.
23. **Kasturi, K. N., R. Mayer, C. A. Bona, V. E. Scott, and C. L. Sidman.** 1990. Germline V genes encode viable motheaten mouse autoantibodies against thymocytes and red blood cells. J. Immunol. **145:**2304–2311.
24. **Kramer, D. R., and J. J. Cebra.** 1995. Early appearance of "natural" mucosal IgA responses and germinal centers in suckling mice developing in the absence of maternal antibodies. J. Immunol. **154:**2051–2062.
25. **Kroese, F. G. M., E. C. Butcher, A. M. Stall, P. A. Lalor, S. Adams, and L. A. Herzenberg.** 1989. Many of the IgA producing plasma cells in the murine gut are derived from self-replenishing precursors in the peritoneal cavity. Int. Immunol. **1:**75–84.
26. **Kroese, F. G. M., J. J. Cebra, M. J. F. van der Cammen, A. B. Kantor, and N. A. Bos.** 1995. Contribution of B1 cells to intestinal IgA production in the mouse. Methods **8:**37–43.
27. **Kroese, F. G. M., R. De Waard, and N. A. Bos.** B1 cells and their reactivity with the murine intestinal microflora. Sem. Immunol. Ser., in press.
28. **Leahy, D. J., G. S. Rule, M. M. Whittaker, and H. M. McConnell.** 1988. Sequence of 12 monoclonal anti-dinitrophenyl spin-label antibodies for NMR studies. Proc. Natl. Acad. Sci. USA **85:**3661–3665.
29. **Logtenberg, T., M. Schutte, S. B. Ebeling, F. H. J. Gmelig-Meyling, and J. H. van Es.** 1992. Molecular approaches to the study of human B cell and (auto)antibody repertoire generation and selection. Immunol. Rev. **128:**23–47.
30. **Mattioli, C., and T. Tomasi.** 1973. The life span of IgA plasma cells from the mouse intestine. J. Exp. Med. **138:**452–460.
31. **McGhee, J. R., J. Mestecky, M. T. Dertzbaugh, J. H. Eldridge, M. Hirasawa, and H. Kiyono.** 1992. The mucosal immune system: from fundamental concepts to vaccine development. Vaccine **10:**75–88.
32. **Michetti, P., M. J. Mahan, J. M. Slauch, J. J. Mekalanos, and M. R. Neutra.** 1992. Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen *Salmonella typhimurium*. Infect. Immun. **60:**1786–1792.
33. **Orlandi, R., D. H. Güssow, P. T. Jones, and G. Winter.** 1989. Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. Proc. Natl. Acad. Sci. USA **86:**3833–3837.
34. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
35. **Pecquet, S. S., C. Ehrat, and P. B. Ernst.** 1992. Enhancement of mucosal antibody responses to *Salmonella typhimurium* and the microbial hapten phosphorylcholine in mice with X-linked immunodeficiency by B-cell precursors from the peritoneal cavity. Infect. Immun. **60:**503–509.
36. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
37. **Seppälä, I., M. Kaartinen, S. Ibrahim, and O. Mäkelä.** 1990. Mouse Ig coded by V_H families S107 or J606 bind to protein A. J. Immunol. **145:**2989–2993.
38. **Sidker, S. K., P. N. Akolkar, P. M. Kaladas, S. L. Morrison, and E. A. Kabat.** 1985. Sequences of variable regions of hybridoma antibodies to alpha- (1-6) dextran in BALB/c and C57BL/6 mice. J. Immunol. **135:**4215–4221.
39. **Silverman, G. J.** 1992. Human antibody responses to bacterial antigens: studies of a model conventional antigen and a proposed model B cell superantigen. Int. Rev. Immunol. **9:**57–78.
40. **Sprent, J., M. Schaefer, M. Hurd, C. D. Surh, and Y. Ron.** 1991. Mature murine B and T cells transferred to SCID mice can survive indefinitely and many maintain a virgin phenotype. J. Exp. Med. **174:**717–729.
41. **Takahashi, S., J. Itoh, M. Nose, M. Ono, T. Yamamoto, and M. Kyogoku.** 1993. Cloning and cDNA sequence analysis of nephritogenic monoclonal antibodies derived from an MRL/lpr lupus mouse. Mol. Immunol. **30:**177–182.
42. **Tucker, P. W., J. L. Slightom, and F. R. Blattner.** 1981. Mouse IgA heavy chain gene sequence: implications for evolution of immunoglobulin hinge regions. Proc. Natl. Acad. Sci. USA **78:**7684–7688.
43. **Van der Heijden, P. J., A. T. J. Bianchi, P. J. Heidt, W. Stok, and B. A. Bokhout.** 1989. Background (spontaneous) immunoglobulin production in the murine small intestine before and after weaning. J. Reprod. Immunol. **15:**217.
44. **Van der Heijden, P. J., and W. Stok.** 1987. Improved procedure for the isolation of functionally active lymphoid cells from the murine intestine. J. Immunol. Methods **13:**161–167.
45. **Van der Heijden, P. J., W. Stok, and A. T. J. Bianchi.** 1987. Contribution of immunoglobulin-secreting cells in the murine small intestine to the 'background' immunoglobulin production. Immunology **62:**551–555.
46. **Van der Waaij, L. A., G. Mesander, P. C. Limburg, and D. Van der Waaij.** 1994. Direct flow cytometry of anaerobic bacteria in human feces. Cytometry **16:**270–279.
47. **Wilkinson, M. H. F., G. J. Jansen, and D. Van der Waaij.** 1994. Computer processing of microscopic images of bacteria: morphometry and fluorimetry. Trends Microbiol. **2:**485–489.
48. **Wold, A. E., C. Motas, C. Svanborg, and J. Mestecky.** 1994. Lectin receptors on IgA isotypes. Scand. J. Immunol. **39:**195–201.

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