

DIFFERENTIATED B LYMPHOCYTES
Potential to Express
Particular Antibody Variable and Constant Regions
Depends on Site of Lymphoid Tissue and Antigen Load*

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Multiple genes code for the variable (V)¹ and constant (C) regions of immunoglobulin polypeptides. In addition to the large number of V genes which code for antibody combining sites, the murine heavy-chain locus has as many as 10 C (C_H) genes which specify molecules with different effector functions. As B cells have the potential to respond to an antigen by producing antibodies with a variety of V and C regions, it is of basic interest to study factors that affect the relative frequencies of certain V region clonotypes within the diverse repertoire, and that determine which C_H isotypes are produced by a B-cell clone after antigen stimulation. We have used the cloning assay of Klinman (1) to study both aspects of B-cell differentiation by examining the influence of lymphoid organ site and antigen load on the expression of V and C regions.

At the level of V region expression, questions about the B-cell repertoire include (a) do various lymphoid tissues have the same frequency of precursors specific for an antigenic determinant, (b) does the repertoire of cells bearing different V regions specific for an antigen vary among tissues, and (c) how do encounters with antigen change the frequency and repertoire of affected B cells? Using a probe for the V region idiootype, the number of cells expressing a particular V region (clonotype) can be assessed relative to the total number of B cells specific for that antigen. Thus both the total number of precursors and the size of a subset within that population can be compared among lymphoid compartments before and after antigen stimulation.

With regard to C_H region expression, recent findings indicate that some B cells have the potential to generate a clone secreting several heavy-chain classes (2, 3). However, our previous observations have suggested that cells from Peyer's patches, which are lymphoid follicles in the walls of the small intestine, generate a high proportion of plasma cells expressing IgA compared with B cells from other tissues (4, 5). We have now quantitatively analyzed B-cell potential at the single cell level in an effort to deduce how the microenvironment of Peyer's patches may influence the development of B cells with the potential to express a particular heavy-chain class.

Materials and Methods

Mice. 5 wk-old BALB/cJ, A/J, and A/He mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. CB-20 mice were raised in our breeding colony, which was initiated

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¹ *Abbreviations used in this paper:* V, variable; C, constant; C_H, constant region of heavy chain; PC, phosphorylcholine; DNP, 2,4-dinitrophenyl; T15, TEPC 15 myeloma; PBS, 0.02 M phosphate-0.15 M sodium chloride buffer, pH 7.2.

with stock from M. Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md. Germfree BALB/c mice were kindly provided by K. Snowden, Small Animal Section, NIH.

Antigens. *Limulus polyphemus* hemocyanin was purchased from Worthington Biochemical Corp., Freehold, N. J. The preparation of phosphorylcholine (PC)-hemocyanin and *Pneumococcus* R36A has been described (6). The preparation of PC-bovine serum albumin was similar to that of PC-hemocyanin. PC-tyrosylglycylglycine-hemocyanin was synthesized by coupling *p*-diazonium phenylphosphorylcholine (7) to *N*-acetyl-L-tyrosylglycyl-glycine BOC hydrazide (Inman's Derivative G) purchased from Biosearch Research Biochemicals, San Rafael, Calif. (8, 9). The PC-tripeptide spacer was then conjugated to hemocyanin (9). The compound had a ratio of 10 mol of PC per 100,000 g of hemocyanin. 2,4-dinitrophenyl (DNP)-conjugated proteins were prepared according to Eisen (10); DNP-hemocyanin contained 10 mol of DNP per 100,000 g of hemocyanin, and DNP-bovine serum albumin contained 25 mol of DNP per mole of protein.

Two derivatives of inulin ($\beta 2 \rightarrow 1$ linked polyfructose)-conjugated protein were used. In one case, cyanogen bromide-activated inulin was coupled directly to hemocyanin. Inulin (8 mg, Calbiochem Inc., La Jolla, Calif.) was dissolved in 2 ml of H₂O by heating, and 8 mg of cyanogen bromide was dissolved in 1 ml of H₂O. The inulin solution was brought to pH 11 with sodium hydroxide; the cyanogen bromide solution was added dropwise to inulin while stirring and the pH was maintained at 11 for 5 min. Then the activated inulin was quickly added to 54 mg of hemocyanin, which was dissolved in 5 ml of 0.07 M sodium borate decahydrate-0.07 M boric acid-0.15 M sodium chloride, pH 8.3 (coupling buffer). The solution was stirred at 4°C overnight and then dialyzed against 0.02 M phosphate-0.15 M sodium chloride, pH 7.2 (PBS). The other antigen, hemocyanin-(*p*-aminophenyl) butyryl-*N*-(2-aminoethyl) carbamyl methylated-inulin was synthesized and graciously provided by C. C. Chien, NIH. Both inulin conjugates contained 1-3 mol of inulin per 100,000 g of hemocyanin.

Immunizations. Mice were immunized intraperitoneally with 0.1 mg of hemocyanin in complete Freund's adjuvant 3 mo before use as recipients in the splenic focus assay. C. Crandall, University of Florida, Gainesville, Fla., inoculated mice with embryonated eggs of *Ascaris suum* by stomach intubation. Anti-T15 and anti-S117 antisera were raised in A/He mice according to Lieberman and Humphrey (11), by using purified proteins as immunogens. Antiserum specific for murine Fab determinants was raised in a goat against pepsin-digested serum IgG, which was purified on DEAE-cellulose (12). The protein (5 mg) in complete Freund's adjuvant was distributed in intradermal sites across shoulders and flanks and in hind quarters. Antibodies to heavy-chain classes were raised in rabbits, which were given 1 mg of myeloma protein in complete Freund's adjuvant distributed in all footpads and many intradermal sites.

Immunoadsorbents. The preparations of PC-bovine serum albumin coupled to bromoacetyl cellulose (6) (Sigma Chemical Co., St. Louis, Mo.), and DNP-lysine coupled to bromoacetyl cellulose (13) have been described. Inulin coupling to bromoacetyl cellulose was accomplished by mixing 2.5 g (wet weight) of bromoacetyl cellulose with 25 mg of hexane diamine and 25 mg of *n*-amino caproic acid, which were both dissolved in 25 ml of H₂O. The mixture was stirred overnight at room temperature and washed three times with PBS by centrifugation. The cellulose was then suspended in 20 ml of coupling buffer. 70 mg of inulin was dissolved in 7 ml of H₂O and brought to pH 11 with sodium hydroxide. 70 mg of cyanogen bromide, dissolved in 2 ml of H₂O, was added dropwise to the inulin solution while stirring, and the pH was maintained at 11 for 5 min. The activated inulin was then immediately added to the aminohexyl derivative of bromoacetyl cellulose while vigorously stirring. The mixture was stirred overnight at room temperature and then washed three times with PBS. The cellulose pellet was stored as a 30% solution (wt/vol) in 10% agamma horse serum in PBS. A 300- μ g portion of inulin-bromoacetyl cellulose was used per assay tube in the radioimmunoassay.

PC-glycyltyrosine-Sepharose 4B was prepared according to Chesebro and Metzger (7). α -1,3 dextran-Sepharose was prepared by reacting 75 mg of α -1,3 dextran (A. Jeanes, USDA, Northern Regional Research Laboratory, Peoria, Ill.) with 0.38 g of cyanogen bromide in 3.5 ml of H₂O at pH 11 for 10 min. The solution was then added to 15 ml of aminohexyl-Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) which was suspended in 25 ml of coupling buffer and stirred overnight at 4°C. The following day, dextran-conjugated Sepharose was washed on a filter with coupling buffer and then added to 1 M ethanolamine at pH 8 for 2 h. The final product was washed alternately four times with 0.1 M sodium acetate-1 M sodium

chloride, pH 4, and 0.1 M sodium borate-1 M sodium chloride, pH 8. *N*-acetyl glucosamine-Sepharose was prepared by adding 130 mg of aminohexyl *N*-acetyl-glucosamine, provided by Y. C. Lee, Johns Hopkins University, Baltimore, Md., to 25 ml of cyanogen bromide-activated Sepharose 4B (14) in coupling buffer. After stirring overnight at 4°C, the gel was washed as described above. DNP-lysine-Sepharose was prepared according to Goetzl and Metzger (15). Myeloma proteins were conjugated to cyanogen bromide-activated Sepharose 4B at a ratio of 1–2 mg of protein per ml of gel by the technique described above.

Myeloma Proteins. Plasmacytomas T15, MOPC 104E, MOPC 460, and MOPC 315 were obtained from M. Potter; S117 was provided by K. Eichmann, Institute for Immunology and Genetics, Heidelberg, F.R.G. T15 (κ, α) ascites fluid was purified on PC-glycyltyrosine-Sepharose as previously described (6). MOPC 104E (λ, μ) ascites fluid was absorbed to dextran-Sepharose and eluted with 0.25 N acetic acid. MOPC 460 (κ, α) and MOPC 315 (λ, α) ascites fluids were mildly reduced and alkylated (15), bound to DNP-Sepharose, and eluted with 0.25 N acetic acid. S117 (κ, α) ascites fluid was absorbed to *N*-acetyl glucosamine-Sepharose and eluted with 1% *N*-acetyl glucosamine, followed by extensive dialysis. MOPC 21 ($\kappa, \gamma 1$) and ADJPC 5 ($\kappa, \gamma 2a$) proteins were supplied by R. Taylor, University of Bristol, England.

Idiotype, Fab, and Isotype-Specific Antibody. A/He antiserum specific for the T15 idiotype was prepared by passing antisera over MOPC 460-Sepharose as described (6). Goat anti-Fab globulin was absorbed to serum IgG, which was purified on DEAE-cellulose and coupled to Sepharose, and the specific antibodies were eluted with 0.2 M propionic acid-0.15 M sodium chloride at 4°C. Purified anti- μ was prepared by passing rabbit antiserum to MOPC 104E over MOPC 315-Sepharose, and then binding anti- μ antibody to MOPC 104E Sepharose and eluting with propionic acid as described above. Anti- $\gamma 1$ was prepared by passing rabbit antiserum to MOPC 21 over ADJPC 5-Sepharose, and then binding the $\gamma 1$ -specific antibody to MOPC 21-Sepharose, followed by acid elution. Anti- $\gamma 2$ was prepared by passing rabbit anti-ADJPC 5 serum over MOPC 21-Sepharose and absorbing $\gamma 2$ -specific antibody to ADJPC 5-Sepharose, followed by acid elution. Anti- α was prepared by passing rabbit antiserum to MOPC 315 over MOPC 104E-Sepharose and absorbing α -specific antibody to MOPC 315-Sepharose, followed by acid elution.

Splenic Focus Assay. Spleen, Peyer's patch, and mesenteric lymph node cell preparations from donor mice were obtained by teasing cells out of the tissue into Hanks' Balanced Salt Solution (Grand Island Biological Co., Grand Island, N. Y.), and filtering the suspension through a 10-ml syringe filled with $\frac{1}{4}$ inch of cotton to remove clumps and epithelial cells. About $4-7 \times 10^6$ Peyer's patch lymphocytes were recovered per mouse from 6–8 patches on the small intestine; only 3×10^6 Peyer's patch cells were obtained per germfree mouse. The following number of donor cells were injected intravenously per lethally irradiated (1,300 rads), hemocyanin-primed recipient: for PC and inulin analysis, Peyer's patches, 5×10^6 , spleen and mesenteric lymph nodes, $15-20 \times 10^6$; for DNP analysis, Peyer's patches 3×10^6 , spleen, 4×10^6 . Fragment cultures were prepared from spleens of recipient mice 16 h after cell transfer (16). Fragments were stimulated in vitro with either PC-tyrosylglycylglycine-hemocyanin (1×10^{-6} M PC), inulin-hemocyanin (1×10^{-7} M inulin), hemocyanin-(*p*-aminophenyl)butyryl-*N*-(2-aminoethyl) carbamyl methylated-inulin (1×10^{-8} M inulin), or DNP-hemocyanin (2×10^{-6} M DNP). The two inulin antigens were indistinguishable both in the frequency of B cells stimulated and in the range of immunoglobulin classes secreted by clones. Culture fluids were changed every 3 days and fluids were collected 9–15 days after stimulation and assayed for antibody, C_H isotype, and idiotype by radioimmunoassay.

Radioimmunoassay. ¹²⁵I-labeled anti-mouse Fab was used to detect antibody bound to PC, DNP, or inulin-bromoacetyl cellulose (12), or to PC- or DNP-bovine serum albumin-coated polyvinyl plates (17). Heavy-chain classes were detected by the binding of ¹²⁵I-labeled rabbit anti- μ , anti- $\gamma 1$, anti- $\gamma 2$, or anti- α to antibody absorbed to antigen-conjugated bromoacetyl cellulose. Antibody with the T15 idiotype was detected by inhibition of ¹²⁵I-labeled T15 protein binding to anti-idiotypic-coated plastic test tubes (6). Antibody with the Ig-2^a allotype was detected by adding a 33% ammonium sulfate fraction of A/He anti-S117 sera to antibody bound to PC-bromoacetyl cellulose, and then adding ¹²⁵I-labeled anti-IgG2.

Fluorescent Staining. Goat anti-Fab was labeled with tetramethyl-rhodamine (18) and used to count the number of immunoglobulin-bearing cells to determine the percent of B cells in a tissue.

Results

Antigen Exposure Influences the Frequency of Anti-PC B Cells with and without the T15 Idiotype. As seen in Table I, B cells specific for PC are found in Peyer's patches in incidences comparable to those found for spleen. The majority of BALB/c splenic B cells specific for PC belong to the T15 clonotype (6). In contrast, the majority of cells from Peyer's patches express non-T15 idiotypes. One explanation for the difference in clonotype expression could be that cells in Peyer's patches are chronically exposed to intestinal bacteria and other antigens containing the PC-determinant (19). These antigens may selectively stimulate clonal expansion of non-T15 precursors, hence quantitative differences in the V region repertoire may be related to the different antigenic environment of cells in Peyer's patches compared to that of cells in spleen.

This notion was tested by examining the frequency of PC-specific precursors from mice in three stages of antigen exposure: germfree, conventionally-reared, and infected orally with *Ascaris suum*. *Ascaris* is a nematode that elicits an anti-PC response in serum after introduction by stomach intubation of embryonated eggs and subsequent migration of worms through the intestine to other parts of the animal (20). The serum anti-PC response to several PC-containing antigens is demonstrated in Table II. Total circulating anti-PC antibody is lowest in germfree mice and highest in immune mice.

TABLE I
*Frequencies of PC-Specific B Cells with and without the T15 Idiotype from Nonimmune Spleen and Peyer's Patches**

Cell source	Total precursors per 10^6 B cells	T15 Precursors per 10^6 B cells	Non-T15 precursors per 10^6 B cells	Precursors with T15 idiotype %
Spleen	23 \pm 11	15 \pm 7	8 \pm 6	65 \pm 17
Peyer's patches	33 \pm 7	13 \pm 6	20 \pm 6	39 \pm 16

* The frequency of precursors is based on a homing and stimulation efficiency of cells in the recipient spleen, which is 4% of the donor cells injected (17), and the percent of Ig-positive cells in each tissue (spleen, 40%, Peyer's patches, 60%). The data represent the analysis of $100-300 \times 10^6$ nonimmune donor cells injected into recipients; the mean and standard deviation of six experiments was calculated.

TABLE II
The T15 Response in Immune Sera

Sera*	Total anti-PC antibody $\mu\text{g/ml serum}$	Antibody with T15 idiotype	Total anti-body with T15 idiotype %
Germfree	15	10	66
Conventional	45	23	50
Infected with <i>Ascaris</i>	267	50	19
Immunized with PC-hemocyanin	181	56	31
Immunized with R36A	280	144	52

* The data represent the average amount of antibody in the sera from six mice for each group. Mice infected orally with *Ascaris* were bled 18 days after infection. Mice immunized intraperitoneally with 0.1 mg of PC-hemocyanin in complete Freund's adjuvant were bled 7 d after immunization. Mice injected intravenously with three doses of 10^9 *Pneumococcus* R36A were bled 7 d later. The majority of anti-PC antibody in all sera was IgM, except that antibody from the PC-hemocyanin immunized group was predominantly IgG1.

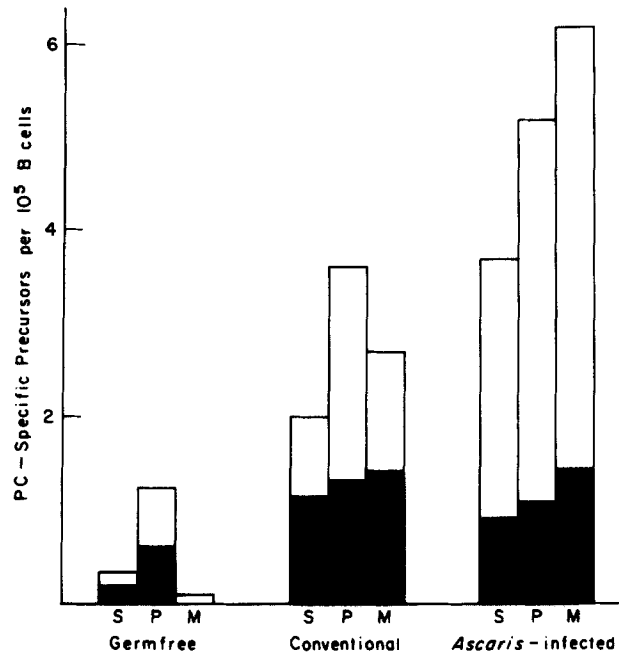


FIG. 1. The effect of antigen on the frequency of PC-specific B cells from several tissues. S, spleen; P, Peyer's patches; M, mesenteric lymph nodes. The shaded area represents T15-positive precursors, and the unshaded area indicates T15-negative precursors. Mice were infected with *Ascaris* by stomach intubation 3–6 mo before analysis. The following number of cells were analyzed for anti-PC precursors: germfree, S, 323×10^6 cells, P, 26×10^6 , and M, 212×10^6 from 21 donors; conventional, S, 475×10^6 , P, 143×10^6 , and M, 122×10^6 from 43 donors; *Ascaris*-infected, S, 108×10^6 , P, 89×10^6 , and M, 40×10^6 from 10 donors. The percent of Ig-positive cells from each tissue was determined by rhodamine-labeled anti-Fab: germfree, S, 32%, P, 46%, M, 23%; conventional and *Ascaris*-infected, S, 40%, P, 60%, M, 30%.

Three types of PC-containing antigens and several routes of administration were tested to compare the percent of anti-PC antibody with the T15 idioype. Only 19% of serum antibody from mice infected orally with *Ascaris* reacts with anti-T15 antibody, compared to 31% from mice injected intraperitoneally with PC-hemocyanin in adjuvant, and 52% from mice injected intravenously with *Pneumococcus* R36A. Brown and Crandall also report that 20% of anti-PC serum antibody from *Ascaris*-infected mice contains the T15 idioype (20). These results show that *Ascaris*, administered orally, preferentially stimulated non-T15 B cells compared to other PC-containing antigens injected intraperitoneally or intravenously.

The amount and idioype composition of antibody found in sera reflects the frequency of B cells specific for PC and percent of these cells with the T15 idioype. The data illustrated in Fig. 1 show that the frequency of total anti-PC precursors from spleen, Peyer's patches, and mesenteric lymph nodes is lowest from germfree donors, intermediate from conventionally-reared mice, and highest from *Ascaris*-infected mice. Lymphoid tissues from germfree mice contain a lower frequency of both T15 and non-T15 precursors than tissues from conventional donors. The percent of total anti-PC clones producing the T15 idioype is substantially lower in all three tissues taken from *Ascaris*-immune mice compared to the percent of clones producing this idioype derived from conventional donors. In fact, the increased frequency of

TABLE III
Distribution of Anti-PC Clones According to C_H Isotypes Expressed

Isotypes expressed by individual clones*	Spleen		Peyer's patches	
	T15-Positive	T15-Negative	T15-Positive	T15-Negative
	% clones‡			
μ	12	5	2	1
γ1	14	7	7	13
α	10	12	45	33
μ,α	24	19	22	16
γ1,α	7	10	11	25
μ,γ1	11	13	2	1
μ,γ1,α	22	34	11	11

* 7% of the foci produced IgG2; antibody with the IgG3 isotype was not determined.

‡ The number of clones analyzed for isotypes was 159 from spleen and 124 from Peyer's patches.

TABLE IV
Increased Expression of IgA by Anti-Inulin Clones Compared to Anti-DNP Clones*

Isotypes expressed by individual clones‡	Anti-inulin		Anti-DNP	
	Spleen	Peyer's patches	Spleen	Peyer's patches
	% clones‡			
μ	22	11	20	16
γ1	11	7	1	11
α	16	41	1	11
μ,α	18	4	17	7
γ1,α	2	11	2	14
μ,γ1	18	15	26	11
μ,γ1,α	13	11	33	30

* The frequency of B cells specific for inulin is 21 per 10⁶ splenic B cells and 23 per 10⁶ Peyer's patch B cells; the frequency of precursors specific for DNP is 56 per 10⁶ splenic B cells and 28 per 10⁶ Peyer's patch B cells. The following number of clones were analyzed for heavy chain class: anti-inulin, spleen, 45, Peyer's patches, 27; anti-DNP, spleen, 88, Peyer's patches, 44.

‡ 12% of anti-inulin clones and 16% of anti-DNP clones expressed IgG2 in combination with another immunoglobulin class; there were no clones secreting only IgG2.

total PC-specific precursors from *Ascaris*-primed donors is due solely to the expanded non-T15 population.

B Cells from Peyer's Patches Generate More Clones Secreting Only IgA than Cells from Spleen. As shown in Table III, individual clones with and without the T15 idiotype from spleen and Peyer's patches of conventionally-reared mice produce IgM, IgG1, and IgA antibodies either singly or in combination. There is no correlation between the size of a clone and the immunoglobulin class expressed (21). The distribution of C_H isotypes expressed by T15-positive and negative clones is comparable within a tissue. A notable difference in isotype expression exists between the two tissues, however. Nearly one-third to one-half of clones from Peyer's patch B cells produced anti-PC antibody with only the α-isotype, compared to 10–12% of clones from splenic B cells. Cells from Peyer's patches that are specific for two other antigenic determinants, inulin and DNP, also generate more clones secreting only IgA relative to cells from spleen (Table IV).

TABLE V
Peyer's Patch B-Cell Commitment to IgA after Long-Term Residence in Congenic Spleens

BALB/c cell source*	Anti-PC clones expressing			
	IgM	IgG1	IgA	IgA donor cell allotype
			%	
Peyer's patches	53	26	95	100
Mesenteric lymph nodes	100	40	40	100
Bone marrow	100	30	0	—

* 10 to 20 × 10⁶ cells from BALB/c tissues were injected intravenously into nine CB-20 recipients irradiated with 450 rads. 1, 4, and 6 d later, the recipients were immunized intravenously with 10⁹ heat-killed cells of *Pneumococcus* R36A to expand the BALB/c cell population. 1 mo later, the recipients' spleen cells were injected into lethally irradiated, hemocyanin-primed BALB/c recipients and stimulated in vitro with PC-tyrosylglycylglycine-hemocyanin.

The disproportionate number of Peyer's patch clones producing only IgA compared to spleen clones does not appear to be due to different B-cell clonotypes within these tissues responding to antigen, since the difference exists between B cells expressing the same V region idiotype (Table III). Both Peyer's patch and splenic-derived B cells are stimulated under identical in vitro conditions accompanied by the same helper T-cell populations. The results suggest that the Peyer's patch B-cell population is qualitatively different from that in spleen based on increased expression of only IgA by derivative clones. Furthermore, cells from Peyer's patches retained the potential to produce clones expressing predominantly IgA even after long-term residence in an intermediate spleen as shown in Table V. Donor cells from BALB/c mice were injected into sublethally-irradiated CB-20 recipients, which are congenic for BALB/c histocompatibility antigens, but differ at the IgC_H locus. 1 mo later, the CB-20 spleens were removed and transferred to BALB/c mice for the splenic focus assay. Nearly all of the PC-specific clones from the Peyer's patch inoculum produced IgA, half of the clones from mesenteric lymph node cells produced IgA, and none of the clones from bone marrow cells made IgA. All of the IgA antibody had the BALB/c donor cell allotype, indicating no host repopulation of the intermediate CB-20 spleen after 1 mo. Therefore the potential of anti-PC B cells from Peyer's patches to produce IgA is not transient and is maintained even after long-term cell transfer.

Clones from Peyer's Patch B Cells Specific for Environmental Antigenic Determinants Express Predominantly IgA. Since cells in Peyer's patches receive continual antigen stimulation from intestinal organisms, it was of interest to determine if B cells specific for environmental antigens generate more clones producing only IgA than cells specific for unusual determinants. The immunoglobulin classes made by clones from cells specific for PC and inulin, which are found on intestinal bacteria (19, 22), were compared with antibody made by clones from precursors specific for DNP, which is presumably a less common determinant. As seen in Tables III and IV, anti-PC and anti-inulin precursors from Peyer's patches produce 33–45% clones expressing only IgA, whereas anti-DNP precursors from this tissue produce just 11% clones making only IgA.

TABLE VI
*Stimulation of Nonimmune PC-Specific B Cells in Syngeneic Vs. Allogeneic Recipients**

Clones expressing	Spleen		Peyer's patches	
	Syngeneic	Allogeneic	Syngeneic	Allogeneic
	<i>clones per 10⁶ injected cells‡ (% of response)</i>			
μ	0.05 (14)	0.01 (23)	0.03 (6)	0.05 (14)
$\gamma 1$	0.01 (3)	<0.005 (<1)	<0.03 (<1)	<0.02 (<1)
α	<0.01 (<1)	0.02 (38)	0.23 (44)	0.24 (72)
μ, α	0.10 (29)	0.005 (8)	0.17 (31)	0.02 (7)
$\gamma 1, \alpha$	0.02 (6)	0.005 (8)	0.03 (7)	<0.02 (<1)
$\mu, \gamma 1$	0.06 (17)	0.005 (8)	<0.03 (<1)	<0.02 (<1)
$\mu, \gamma 1, \alpha$	0.11 (31)	0.01 (15)	0.07 (12)	0.02 (7)

* Syngeneic transfer: BALB/c cells injected into BALB/c carrier-primed recipients, and A/J cells injected into A/J carrier-primed recipients. Allogeneic transfer: BALB/c cells transferred to A/J carrier-primed recipients, and A/J cells transferred to BALB/c carrier-primed recipients.

‡ 103×10^6 spleen cells were injected into syngeneic recipients, and 204×10^6 spleen cells were transferred to allogeneic recipients. The Peyer's patch cell transfer involved the analysis of 30×10^6 cells transferred to syngeneic recipients and 41×10^6 cells transferred to allogeneic recipients.

If bacterial antigens cross the dome epithelium of Peyer's patches, lymphocytes specific for these antigens may be chronically in a naturally primed state. One criterion for demonstrating the secondary nature of B cells is their capability for stimulation in the splenic focus assay in histoincompatible recipients to produce C_H isotypes other than IgM (23). The data in Table VI show that PC-specific B cells from nonimmune spleen and Peyer's patches produce predominantly IgA-only clones when stimulated with antigen in the presence of irradiated, carrier-primed, histoincompatible T-cells. The minor population of spleen cells that generate IgA-only clones may have migrated from Peyer's patches. The frequency of stimulation of Peyer's patch precursors for IgA-only clones is identical in both allogeneic and syngeneic recipients, whereas the frequencies of most other types of clones is much lower when stimulated in allogeneic fragments compared to syngeneic fragments. The results suggest that precursors of IgA-only clones have different triggering requirements than precursors of clones secreting other combinations of isotypes, observed in our studies of cells from untreated, conventionally-reared mice.

Discussion

An animal responds to an antigenic determinant with a diverse array of antibody molecules (24, 25). To determine if the repertoire of clonotypes with the same nominal specificity is different from organ to organ, we have used an anti-idiotypic antibody that identifies a subset of B cells to characterize antigen-specific cell populations. The majority of anti-PC precursors in BALB/c mice belong to the T15 clonotype, although cells expressing non-T15 idiotypes may represent greater than 200 clonotypes (26). In this report we have shown that precursors for anti-PC antibody with and without the T15 idiomorph reside in gut-associated lymphoid tissues as well as spleen. However, the repertoire of clonotypes differs between these organs in that a lower percent of PC-specific B cells from Peyer's patches express the T15 idiomorph than cells from spleen.

Variation in the V region repertoire in a particular organ could be due to differential migration and lodging of B cells with particular specificities from the stem cell pool in fetal liver and bone marrow, or to local antigen selection and expansion of cells expressing a particular antigen combining site after cell migration and lodging. Regarding the latter possibility, we have found that different PC-containing antigens, administered by alternate routes of immunization, selectively stimulate certain B cells within the PC-specific repertoire. The anti-PC B-cell population from mice infected 3–6 mo previously with *Ascaris* contains an expanded non-T15 precursor population compared to nonimmune or germfree mice, in which the T15 clonotype is prevalent. Thus differences in the repertoire of clonotypes between nonimmune lymphoid organs may indicate different environmental antigenic stimuli for each organ.

In particular, Peyer's patches are located on the small intestine and receive chronic antigenic stimulation from organisms in the gut. Intestinal organisms, like *Ascaris*, may preferentially stimulate anti-PC precursors expressing idiotypes other than T15. The low frequency of anti-PC precursors in Peyer's patches, mesenteric lymph nodes, and spleens from germfree mice is probably due to a lower level of stimulation from the germfree intestinal tract. This study, which shows that gut-associated tissues contain an increased number of antigen-specific B cells several months following oral infection with *Ascaris*, and other reports (27–29) demonstrate the efficacy of oral administration of vaccines to immunize against intestinal pathogens. Perturbations in the V-gene repertoire of a B-cell population may persist long after serum antibody levels subside.

Variation in C_H gene expression by B cells from different tissues was examined by the analysis of three isotypes produced by clones of antigen-stimulated cells. Although B cells from both spleen and Peyer's patches can generate clones producing IgM, IgG1, and IgA singly and in all combinations, cells from Peyer's patches generate more clones synthesizing only the α isotype than cells from spleen. IgA expression by Peyer's patches is increased whether (a) the precursors are specific for PC, inulin, or DNP, (b) the PC-specific clones express the T15 or non-T15 idiotypes, and (c) the cells come from germfree, conventional, or immune animals. Long-term transfer of Peyer's patch cells into the spleens of intermediate hosts did not affect the ability of these cells to produce IgA after antigen stimulation. Expression of the α -isotype is presumably not dependent on T cells specific for the C_a region since both PP and spleen cells are stimulated in the same carrier-primed splenic fragment environment. The data suggest that Peyer's patch precursors of IgA-only clones are qualitatively different from most splenic B cells. Thus, many Peyer's patch lymphocytes of certain specificities are precommitted to generating IgA-expressing progeny.

Preferential commitment to IgA by B cells in Peyer's patches may be explained by considering both an undefined organ-specific role of Peyer's patches and the antigenic environment of cells in this tissue. A role for antigen is suggested by the results that B cells specific for PC and inulin determinants, which are found on intestinal bacteria, generate more clones secreting only IgA than do B cells specific for DNP. This striking correlation between IgA expression and V region specificity for antigen implies that environmental antigens have selected and expanded certain B cells in Peyer's patches which then have the ability to generate progeny that express IgA. Evidence supporting the secondary nature of IgA precursors specific for environmental antigens was obtained using a variation of the splenic focus assay. Experiments by Pierce and Klinman (23) have shown that secondary B cells from spleen, but not primary cells

from the same source, can produce IgG1 after stimulation in histoincompatible spleen fragments from carrier-primed recipients. By analogy, the expression of IgA by many Peyer's patch B cells stimulated in histoincompatible fragments suggests that IgA precursors from conventionally-reared, but not deliberately-immunized mice are secondary cells. Although the donor mice were not immunized, environmental antigens may naturally expand and prime certain B cells from within the nonimmune repertoire.

Several models could account for the high frequency of IgA precursors in Peyer's patches. First, cells arising in a variety of lymphoid organs could randomly become committed to IgA expression, migrate, and selectively lodge in Peyer's patches. Specific cells would then be clonally expanded by gut antigens. Second, a particular V_H gene could be translocated with a higher frequency to the C_α gene than to other C_H genes. Selection for such V_H genes specific for gut-associated antigens could have occurred over evolutionary time, followed by antigen-driven clonal expansion in Peyer's patches during the lifetime of each individual. Nonrandom translocation of a V gene to a particular C gene is not likely, though, since the T15 V_H gene is associated equally frequently with μ , $\gamma 1$, and α - C_H genes by clones from splenic-derived B cells. The predominant expression of IgA by T15 precursors in Peyer's patches suggests that this organ plays a critical role in favoring the expression of the C_α gene. Third, IgA expression may be related to the number of antigen-induced divisions a cell has undergone. As recent evidence (30) indicates that the C_α gene is farthest from the V genes, only the most differentiated B cells would express IgA if V-gene translocation to C genes beyond C_μ and C_δ -genes is orderly. However, reports that cells from neonatal animals express IgA on their membrane (31) and that C_H -isotype expression is not sequential within a clone developing in vitro (32) would argue against the division-dependent model of IgA expression. Fourth, Peyer's patches could provide a special milieu, such as the presence of a subpopulation of T cells (33), the lack of an organized antigen-trapping reticulum (34), or a hormone, which influences the differentiation of immature B cells to become IgA precursors. The microenvironment could influence the generation of IgA precursors in situ by a mechanism coupled with antigen-driven cell division.

Summary

B cells have the potential to respond to an antigen by producing antibodies with a variety of variable and constant regions. We have quantitatively analyzed B-cell potential at the single cell level to determine the effect of lymphoid tissue site and antigen load on the expression of variable and constant regions. Concerning variable region expression, although the total frequency of B-cell precursors for phosphorylcholine is similar between nonimmune spleen and gut-associated Peyer's patch tissues, the proportion of cells producing non-TEPC 15 idiotypes is greater from Peyer's patch than from spleen. Oral immunization with phosphorylcholine-containing *Ascaris suum* increased the frequency of non-TEPC 15 B cells. Thus variation in the proportion of cells bearing different variable regions may be related to the distinct antigenic environment of cells in Peyer's patches compared to that of cells in spleen.

Regarding constant region expression, although B cells from both spleen and Peyer's patches generate clones producing IgM, IgG1, and IgA singly and in all combinations, cells from Peyer's patches generate more clones secreting only IgA than cells from spleen. B cells specific for phosphorylcholine and inulin, which are found

on intestinal bacteria, produce more IgA-only clones than B cells specific for the dinitrophenyl determinant. This striking correlation between IgA expression and variable region specificity for antigen implies that environmental antigens have expanded certain B cells in Peyer's patches which then have the ability to generate progeny that express only IgA. Evidence supporting the secondary nature of precursors for IgA-only clones is obtained by their ability to produce this isotype after stimulation with histoincompatible T cells. The role of gut antigens may be to clonally expand IgA precursors and perhaps to stimulate the proliferation of less differentiated cells within the unique microenvironment of the Peyer's patches, allowing them to differentiate to IgA precursors.

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