

# RESTRICTION OF GENE EXPRESSION IN B LYMPHOCYTES AND THEIR PROGENY

## III. Endogenous IgA and IgM on the Membranes of Different Plasma Cell Precursors\*

BY PATRICIA P. JONES‡, § AND JOHN J. CEBRA

(From the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218)

During their differentiation from stem cells to mature plasma cells bone-marrow or bursa-derived (B) lymphocytes become committed to the synthesis of a single molecular species of Ig (reviewed in reference 1). The commitment to heavy and light chain variable regions probably occurs relatively early in the evolution of B lymphocytes from stem cells, thus determining the specificity of the antigen-binding site expressed by the cell and its progeny (2, 3), but the stage at which lymphoid cells become committed with respect to the isotype of their Ig product is not definitely known. A variety of studies in which Ig synthesis has been inhibited by treatment with anti- $\mu$ -chain antibodies and/or bursectomy has suggested that B-lymphocyte precursors for plasma cells synthesizing IgA, IgG, and IgM may all be derived from IgM-bearing lymphocytes (4-6). After exposure to antigen, lymphocytes might then begin to express membrane receptors of the same isotype as the molecules which will be produced by their plasma cell progeny (7-9).

In studies of the precursor potential of lymphocytes from a variety of tissues in the rabbit, we found that both the Peyer's patches and the appendix, two gut-associated lymphoid tissues (GALT),<sup>1</sup> are enriched sources of precursors of IgA-producing plasma cells (reference 10 and footnote 2). Lymph nodes and peripheral blood, on the other hand, give rise mainly to plasma cells synthesizing IgG and IgM. When purified populations of Peyer's patch lymphocytes were isolated with a fluorescence-activated cell sorter (FACS) on the basis of their membrane Ig markers and then allowed to differentiate into plasma cells, the precursors of the IgA-producing plasma cells were found to be  $\mu$ -, b+ lympho-

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§ Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, Calif. 94143.

<sup>1</sup> *Abbreviations used in this paper:* F, fluorescein; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; GALT, gut-associated lymphoid tissue; R, rhodamine.

<sup>2</sup> Craig, S. W., and J. J. Cebra. 1974. Rabbit Peyer's patch, appendix, and popliteal lymph node B lymphocytes: a comparative analysis of their membrane immunoglobulin components and plasma cell precursor potential. Manuscript submitted for publication.

cytes, having light chain markers determined by the *b* locus but no detectable  $\mu$ -chains (11). At that time, attempts to detect other endogenous heavy chains on these IgA precursors using heterologous anti- $\alpha$  or anti- $\gamma$  antisera were unsuccessful.

The present paper reports the identification of  $\alpha$ -chains on the membranes of these  $\mu$ -,*b*+ lymphocytes. Their detection was made possible by the use of antibody reagents specific for the *f* and *g* locus allotype markers occurring, respectively, on the  $\alpha_1$ - and  $\alpha_2$ -chains of the two IgA subclasses (12). Further staining analyses revealed that the GALT contains relatively high proportions of  $\alpha$ -bearing cells, consistent with their being enriched sources of immediate precursors of IgA-producing plasma cells.

## Materials and Methods

*Rabbits.* Several of the rabbits used as cell donors were kindly provided by Doctors K. L. Knight of the University of Illinois Medical School, Chicago, Ill., and R. G. Mage, NIAID, NIH, Bethesda, Md.; others were raised in our own facilities. Serum samples from some of these animals were typed for *f* and *g* locus  $\alpha$ -chain allotypes by Dr. K. L. Knight.

*Antibody Reagents.* The preparation and specificity of rhodamine (R)- and fluorescein (F)-conjugated anti-Ig reagents have been described elsewhere (reference 13 and footnote 2). Specific rabbit anti-*f* and *g* locus reagents (anti-*f,g*) were a generous gift from Dr. K. L. Knight. The preparation of anti-*f*71,*g*75; anti-*f*72,*g*74; and anti-*f*73,*g*74 antisera have been described (14). Sensitive radiomunoprecipitation assays showed that these antisera do not recognize determinants on IgG molecules (K. L. Knight, personal communication), nor do they have significant reactivity for secretory component (15). To lessen the remote possibility that there might still be undetected antibodies present reacting with non- $\alpha$ -chain sIgA determinants, such as secretory component or J chain, the anti-*f*71,*g*75; anti-*f*72; and anti-*g*74 reagents supplied to us were passed through appropriate sIgA Sepharose-4B immunoabsorbent columns. The anti-*f*72 reagent was prepared by passing anti-*f*72,*g*74 antiserum through an immunoabsorbent column of *f*71,*g*75 and *f*73,*g*74 sIgA, and the anti-*f*71,*g*75 had been passed through an *f*72,*g*74 and *f*73,*g*74 sIgA column. The anti-*g*74 antiserum was passed through immunoabsorbent columns of *f*72, *f*73, and *f*71,*g*75 sIgA; the *f*72 and *f*73 sIgA had been prepared from the pool I obtained by the gel filtration of papain digests of *f*72,*g*74 and *f*73,*g*74 sIgA, respectively (15). Additional specificity controls will be presented later.

Because rabbit IgM binds nonspecifically to rabbit lymphocyte membranes,<sup>3</sup> only IgG fractions of the anti-*f* and *g* allotype antisera were used for membrane staining. IgG fractions of the anti-*f*72; anti-*f*72,*g*74; and anti-*f*73,*g*74 sera were prepared by Dr. Knight; fractionations of the anti-*g*74 and anti-*f*71,*g*75 sera were performed in our laboratory.

*Preparation of Cells.* Single cell suspensions were prepared as previously published (11, 13). In some experiments, cells were stripped of membrane Ig with pronase and cultured overnight at 37°C to remove exogenous membrane Ig and to permit the cells to regenerate their own membrane Ig, as described earlier (13, as modified in reference 16).

*Membrane Staining* Lymphocytes were membrane stained as previously described (13), using fluorescent antibody reagents at concentrations giving optimal staining. A two-step (indirect) staining procedure was used to detect *f* and *g*  $\alpha$ -chain allotype markers on cell membranes. The cells were first incubated in the IgG antiallotype reagents, washed, and then incubated in R-anti- $\gamma$  to stain bound IgG molecules.

Smears of membrane stained cells were examined with a Zeiss RA Standard microscope (Carl Zeiss, Inc., New York) equipped for transmitted light fluorescence with a 47 25 10 lamp housing, a 200 W high pressure mercury arc lamp (type 202, Illumination Industries, Inc., Sunnyvale, Calif.), and a 200 W DC short arc power supply (Baltimore Instruments Co., Inc., Baltimore, Md.). The filters used for exciting and detecting R and F fluorescence have been described (10) except for a 500

<sup>3</sup> Jones, P. P., and J. J. Cebra. Manuscript in preparation.

nm Zeiss yellow barrier filter (Baltimore Instruments Co. Inc.) used for examining F fluorescence. For cell preparations which had been stained with only a R-labeled reagent, 500 cells were counted/slide. For preparations stained with both F and R reagents, 300 cells were examined for the presence of R and/or F membrane staining.

## Results

*Detection of f and g  $\alpha$ -Chain Allotypic Determinants on Lymphocyte Membranes.* The proportions of lymphocytes bearing IgA markers on their membranes were determined for cells from various lymphoid tissues. Because earlier studies had shown that rabbit lymphocytes bind serum Ig molecules (13), in some experiments cells were treated with pronase to remove all membrane Ig and then cultured overnight to allow them to re-express their own endogenous membrane Ig molecules before they were stained. Since the f and g markers are on different molecules, an anti-f,g reagent will stain cells with the f marker only, the g marker only, and cells with both markers if such cells are present. Cytoplasmic staining with anti-f and anti-g reagents has revealed that the two subclasses of IgA are synthesized by different plasma cells (17). In Table I are presented the results of staining cells from f71,g75 rabbits, and Table II contains the results for f72,g74 and f73,g74 rabbits. Although the percentages of positive cells vary considerably among the nine animals studied, the proportions of cells bearing f or g  $\alpha$ -chain markers are consistently higher for the GALT than for the other lymphoid tissues.

This difference is most apparent when one examines the results obtained with cells which had been stripped with pronase and then cultured so that Ig molecules of exogenous origin were removed from the membrane. In the four rabbits in which both lymph node cells and GALT cells were stained after stripping and culture, the proportions of cells with f,g markers were 5.7 (rabbit 4), 60 (rabbit 7), 10 (rabbit 8), and 31 (rabbit 9, using the anti-f73,74 reagent) times higher in the GALT (averaging the percentages for Peyer's patch and

TABLE I  
*f and g  $\alpha$ -Chain Allotype Determinants on Membranes of Lymphocytes from f71,g75 Rabbits\**

Tissue	% Stained for f71, g75					
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6
Peyer's patch	(24) 12‡	(44) 9.8	8.1	(8.8) 9.0	(21) 50	(17)
Appendix				9.2		(16) 9.3
Peripheral blood				(5.0)§		
Lymph node				1.6		(12)
Spleen						(3.6) 2.0

\* All rabbits were f<sup>71</sup>g<sup>75</sup>/f<sup>71</sup>g<sup>75</sup> homozygotes.

‡ Numbers inside parentheses are results from staining cells not previously stripped with pronase; numbers outside parentheses are results from staining cells after stripping with pronase and overnight culture.

§ Staining of peripheral blood cells was done 3 wk before the animal was killed and the cells from the other tissues were stained.

TABLE II  
*f* and *g*  $\alpha$ -Chain Allotype Determinants on Membranes of Lymphocytes from *f72,g74* and *f73,g74* Rabbits

Tissue	Rabbit 7 ( <i>f72g74</i> / <i>f73g74</i> )				Rabbit 8 ( <i>f72g74</i> /?)		Rabbit 9 ( <i>f73g74</i> / <i>f73g74</i> )		
	% Stained for				% Stained for		% Stained for		
	<i>f72</i>	<i>g74</i>	<i>f72,g74</i>	<i>f73,g74</i>	<i>g74</i>	<i>f72,g74</i>	<i>g74</i>	<i>f72,g74</i>	<i>f73,g74</i>
Peyer's patch	19*	8.4	(49) 40	18	5.2	29	27	29	54
Appendix	16	6.4	(46) 32	15		18	42	30	(51) 44
Peripheral blood	(6.3)‡	(0)	(25)	(8.0)			(10)	(10)	(20) 14
Lymph node			0.6			2.4		2.0	
Spleen			7.2						

\* Numbers inside parentheses are results from staining cells not previously stripped with pronase; numbers outside parentheses are results from staining cells after stripping with pronase and overnight culture.

‡ Staining of peripheral blood cells was done 2 wk before the animal was killed and cells from the other tissues were stained.

appendix) than in the lymph nodes. Similarly, in rabbits no. 6 and 7 the GALT had, respectively, 4.6 and 5.0 times higher a proportion of *f,g*-stained cells than did the spleen. In rabbit no. 9, a considerably higher percentage of cells bore *f* and *g* determinants in the peripheral blood (14%) than in the lymph nodes (1.6%), but still there was a 3.5-fold greater percentage in the GALT compared to the peripheral blood. Thus, in the Peyer's patches and appendix, tissues shown to be enriched in precursors of IgA-producing plasma cells (reference 10 and footnote 2), a significantly higher proportion of total lymphocytes had  $\alpha$ -chain markers on their membranes than did cells from nongut-associated lymphoid tissues.

As a verification of the allotypic specificity of the anti-*f,g* reagents, some cells in the experiments just described were incubated in antibody reagents directed against allotypes other than the animals' own. Very few cells, if any, stained for allotypic specificities not corresponding to the genotype of the cell donor (Table III). In addition to demonstrating specificity for  $\alpha$ -chain markers, these negative control experiments clearly show that the R-anti- $\gamma$  does not stain cells by itself and that neither the antiallotype reagents nor the R-anti- $\gamma$  second step recognizes non-Ig determinants on the membranes of lymphocytes.

The data indicate that both *f* and *g* locus allotypic specificities, on the  $\alpha$ -chains of IgA<sub>1</sub> and IgA<sub>2</sub>, respectively, are detectable on the membranes of rabbit lymphocytes. As shown in Table II, both the anti-*f72* and the anti-*g74* reagents reacted with membrane molecules; the anti-*f72,g74* and anti-*f73,g74* reagents, which have combined activities for the two types of chains, stained more cells than did either the anti-*f72* or the anti-*g74* reagents alone, suggesting that cells bear IgA molecules of only one subclass on their membranes.

*Demonstration that Cells with Membrane  $\alpha$ -Chain Markers are  $\mu$ -, *b*+ Lymphocytes.* We have previously shown that the Peyer's patch precursors of IgA-producing plasma cells have *b* allotype  $\kappa$ -chain markers on their membranes but no detectable  $\mu$ -chains (11). The finding that there are cells in the Peyer's patches and appendix which have *f,g* determinants made it seem likely that the unidentified heavy chain on these  $\mu$ -, *b*+ cells was the  $\alpha$ -chain. To obtain

TABLE III  
Control Membrane Staining for Specificity of Anti-f and g Reagents

Rabbit	Allotype	Tissue	% Stained for						
			f71,g75	f72	g74	f72,g74	f73,g74		
1	f <sup>71</sup> g <sup>75</sup> /f <sup>71</sup> g <sup>75</sup>	Peyer's patch				(0)	0*	(0)	0
2	f <sup>71</sup> g <sup>75</sup> /f <sup>71</sup> g <sup>75</sup>	Peyer's patch				(0)	0	(0)	0
3	f <sup>71</sup> g <sup>75</sup> /g <sup>71</sup> g <sup>75</sup>	Peyer's patch			0.6				
4	f <sup>71</sup> g <sup>75</sup> /f <sup>71</sup> g <sup>75</sup>	Peyer's patch			0		0.2		0.2
		Peripheral blood			0				
5	f <sup>71</sup> g <sup>75</sup> /f <sup>71</sup> g <sup>75</sup>	Peyer's patch			(0)				0
6	f <sup>71</sup> g <sup>75</sup> /f <sup>71</sup> g <sup>75</sup>	Peyer's patch					(0.2)		(0.2)
		Appendix		0.2	0.2				
7	f <sup>72</sup> g <sup>74</sup> /f <sup>72</sup> g <sup>74</sup>	Peyer's patch	(0)						
		Peripheral blood	(1.0)						
9	f <sup>73</sup> g <sup>74</sup> /f <sup>73</sup> g <sup>74</sup>	Peyer's patch	0.2	0					
		Appendix		0					
		Peripheral blood	(0.5)	(0)					

\* See footnote to Tables I and II.

evidence on this point, lymphocytes from a variety of tissues were stained after pronase stripping and subsequent culture with two combinations of reagents. The first, F-anti- $\mu$  and R-anti-b5, would inform us about the proportions of cells which were  $\mu$ -, b+ in b<sup>5</sup>/b<sup>5</sup> rabbits. The second set of reagents, F-anti- $\mu$  and anti-f,g followed by R-anti- $\gamma$ , would reveal the proportions of cells with membrane  $\alpha$ -chains and would also indicate whether the  $\alpha$ - and  $\mu$ -chains occurred on the same cells. The results of these stainings are given in Table IV. For Peyer's patch and appendix cells the proportion of  $\mu$ -,  $\alpha$ + cells was usually within a few percent of the proportions of  $\mu$ -, b+ cells, except for rabbit no. 5. The explanation for the greater numbers of  $\mu$ -,  $\alpha$ + than of  $\mu$ -, b+ cells in this rabbit is not known; one possibility is that it had more  $\alpha$ -bearing cells than usual carrying membrane Ig with  $\lambda$ -type light chains, which lack the b locus allotypes.

Table IV also shows that very few lymphocytes were detected which had been stained for both  $\mu$ -chain and  $\alpha$ -allotype determinants, indicating that these two heavy chains are present on the membranes of different cell populations. The absence of cells double stained for  $\alpha$ - and  $\mu$ -chains also verifies that our anti-f,g allotype reagents do not react with membrane IgM.

For lymph node and spleen cells there are greater differences between the percentages of cells which were  $\mu$ -, b+ and  $\mu$ -,  $\alpha$ + than there are for cells from Peyer's patches and appendix. The proportions of  $\mu$ -, b+ cells in lymph nodes and spleen are considerably closer to the levels of those cells in the Peyer's patches and appendix than are the proportions of  $\mu$ -,  $\alpha$ + cells. Thus there may

TABLE IV  
 $\mu$ -Chain and b, f, and g Allotype Determinants on Membranes of Lymphocytes after  
 Pronase Stripping and Overnight Culture

Rabbit*	Tissue	% Stained‡					
		$\mu+$ ,b+	$\mu+$ ,b-	$\mu-$ ,b+	$\mu+$ , $\alpha+$	$\mu+$ , $\alpha-$	$\mu-$ , $\alpha+$
3	Peyer's patch	32	0	8.0	ND§	ND	8.1
4	Peyer's patch	44	0.3	8.3	0	34	9.7
	Appendix	35	0.3	7.0	0	34	8.3
	Lymph node	55	0.67	5.0	0.3	44	1.3
5	Peyer's patch	10	0	34	0	6.3	50
6	Appendix	36	0.3	7.0	0	31	9.3
	Spleen	39	0	4.3	0.3	36	1.7
8	Peyer's patch	ND	ND	ND	0	34	32
9	Peyer's patch	18	0	51	ND	ND	54
	Appendix	12	0	42	ND	ND	44
	Lymph node	28	1.0	9.7	ND	ND	1.6

\* Rabbits 3-6 were b<sup>6</sup>/b<sup>5</sup>, f<sup>71</sup>g<sup>75</sup>/f<sup>71</sup>g<sup>75</sup>; rabbit 8 was b<sup>6</sup>/b<sup>6</sup>, f<sup>72</sup>g<sup>74</sup>/f<sup>72</sup>g<sup>74</sup>?; rabbit 9 was b<sup>5</sup>/b<sup>5</sup>, f<sup>73</sup>g<sup>74</sup>/f<sup>73</sup>g<sup>74</sup>.

‡ Samples of cells were stained either with both F-anti- $\mu$  and R-anti-b5, or with F-anti- $\mu$ , anti-f,g (specific for the allotype of the cell donor), and R-anti- $\gamma$ .

§ ND, not determined.

|| These samples were stained with anti-f,g followed by R-anti- $\gamma$  but not with F-anti- $\mu$ .

be some b+ cells without either  $\mu$ - or  $\alpha$ -chains in spleen and lymph node but not in the GALT; these cells may bear nondetected  $\gamma$ -chains on their membranes.

*Double Staining for  $\mu$ -Chain and f,g Allotype Determinants before and after Stripping.* A comparison of the results of staining lymphocytes for f,g markers before and after pronase stripping and culture reveals that in some of the rabbits there was a substantial drop in the proportions of total cells which stained for f and g  $\alpha$ -chain markers on their membranes (Tables I and II). While conceivably this could be due to selective losses of IgA-bearing lymphocytes during the overnight culture which follows treatment with pronase, another possibility is that some of the IgA initially found on the cell surfaces had been picked up in situ and did not reflect the cells' own synthetic potential. The binding of serum Ig molecules to rabbit lymphocytes was first noticed during our studies of cells from rabbits heterozygous at the b locus (13). Substantial numbers of lymphocytes initially double stained for both parental b allotypes on their membranes. However, after pronase stripping, only one allotype reappeared on these cells during culture, suggesting that cells were capable of synthesizing molecules of only one allelic allotype at a time. It was subsequently found that serum Ig molecules can bind to lymphocyte membranes, explaining the occurrence of double allotype-staining cells (15).

Similar studies were performed to determine whether some of the anti-f,g staining might be due to the presence of cytophilic Ig on lymphocyte membranes.

Lymphocytes were stained for both  $\mu$ -chains and  $\alpha$ -chain allotype markers as before, either (a) without prior treatment, (b) immediately after pronase stripping, or (c) after pronase stripping and overnight culture (Table V). In cell preparations not treated with pronase, between 2.3 and 14% of total cells were in fact double stained for both heavy chains. However, if the cells were stripped with pronase and cultured to allow them to express their own membrane Ig, very few cells reappeared that could be double stained. (cf. also Table IV, rabbits nos. 4, 5, and 8, for which no double-stained cells were found). Thus the vast majority of lymphocytes do not appear to be able to synthesize both IgA and IgM membrane Ig, at least during the culture period. Those freshly isolated cells which stain for both IgA and IgM may have adsorbed molecules of one or the other isotype onto their membranes while in the animal. Recent studies have in fact shown that both IgM and IgA in rabbit serum can bind to rabbit lymphocyte membranes.<sup>2, 3</sup>

Table V contains an additional point of interest, namely, if Peyer's patch or appendix cells are stained after pronase treatment but before they have been cultured to allow resynthesis of membrane Ig, nearly all the detectable  $\mu$ -chain determinants are gone but significant proportions of cells still stained for f,g  $\alpha$ -chain markers. These results are in accord with our previously published findings: treatment with pronase removes all of the *b* locus  $\kappa$ -chain allotype (13, 16) and  $\mu$ -chain (11) determinants recognized by our reagents, but  $\alpha$ -chains can

TABLE V  
 *$\mu$ -Chain and f and g  $\alpha$ -Chain Allotype Determinants on Membranes of Untreated, Pronase-stripped, and Pronase-stripped and Cultured Lymphocytes*

Rabbit	Tissue	Treatment of cells	% Stained*		
			$\mu+, \alpha+$	$\mu+, \alpha-$	$\mu-, \alpha+$
6	Peyer's patch	None	3.3	13	14
		Lymph node	7.0	18	4.7
	Appendix	None	8.3	15	8.0
		Stripped	0	0	0.2
		Stripped, cultured	0	31	9.3
		Spleen	None	2.3	37
	Spleen	Stripped	0	1.3	0
		Stripped, cultured	0.3	36	1.7
7	Peyer's patch	None	9.7	6.3	39
		Stripped	0	0.3	15
	Appendix	None	12	5.7	34
		Stripped	0	0.7	10
9	Appendix	None	14	2.3	37
		Stripped	0	0.5	5.0
		Stripped, cultured	ND	ND	44‡

\* Samples of cells were stained with F-anti- $\mu$ , anti-f,g (specific for the allotype of the cell donor), and R-anti- $\gamma$ .

‡ This sample was stained with anti-f73,g74 followed by R-anti- $\gamma$  but not with F-anti- $\mu$ .

still be detected by staining cells with purified goat anti-rabbit  $\alpha$ -chain antibodies followed by R-labeled rabbit anti-goat IgG.<sup>2</sup>

## Discussion

With the use of antibody reagents specific for the *f* and *g* locus allotypic determinants present on rabbit  $\alpha_1$ - and  $\alpha_2$ -chains, respectively, we have been able to detect endogenous membrane IgA on the membranes of some rabbit lymphocytes. The ability to detect membrane Ig with these reagents contrasts with the results of earlier experiments in which attempts to detect endogenous membrane IgA on rabbit Peyer's patch cell membranes using purified goat antirabbit  $\alpha$ -chain antibodies were unsuccessful.<sup>2</sup> Although in those studies IgA was found on 43–61% of freshly isolated Peyer's patch cells by indirect immunofluorescence, after the cells had been stripped with pronase and then cultured overnight no IgA was detectable. As we have previously suggested (reference 11 and footnote 2), the lack of membrane staining by the goat anti- $\alpha$  reagent might be due to its activity being directed predominantly against determinants in the Fc portions of the  $\alpha$ -chain which may be buried in the membrane. Since antigenic determinants controlled by the *g* locus and perhaps also by the *f* locus are present in both the Fd and the Fc portions of the  $\alpha$ -chains (12, 15), they should be available to the antibody. Similar observations on the relative availabilities of different determinants on heavy chains have been made for membrane-associated  $\gamma$ -chains (18, 19).

It is interesting to note that if Peyer's patch or appendix lymphocytes are stained with anti-*f, g* immediately after pronase stripping, a definite though diminished population of stained cells can be found (Table V), whereas very few cells stain for  $\mu$ -chain or *b* locus markers on  $\kappa$ -chains (Table V, and references 11, 13, 16). Similar findings were made with the specific goat anti- $\alpha$  reagent;<sup>2</sup> however, after the pronase-stripped cells had been cultured overnight, membrane IgA was no longer detectable with this heterologous reagent. These observations suggest that although pronase digests away the light chain and  $\mu$ -chain antigenic moieties with which the reagents react, enough of the  $\alpha$ -chain is left to result in some staining with our anti-*f, g*. By also removing other membrane components, pronase digestion additionally may expose normally buried determinants on the Fc of the  $\alpha$ -chain, allowing the goat anti- $\alpha$  to react. But as the cells regenerate their coat of membrane molecules during the overnight culture, these determinants may once again become hidden.

By double-staining lymphocytes with a variety of combinations of R- and F-labeled reagents (Table IV), we have been able to show that the previously unidentified heavy chain on the membranes of the  $\mu$ -, *b*+ Peyer's patch cells, the lymphocyte precursors of IgA-producing plasma cells (11), is almost certainly the  $\alpha$ -chain. For formal proof of this conclusion the *f, g*-stained cells would have to be isolated with the FACS and then allowed to differentiate into plasma cells whose product could be examined, as was done with the FACS-separated  $\mu$ -, *b*+ cells (11). However, on the basis of three sets of observations it seems very likely that most of the  $\mu$ -, *b*+ Peyer's patch cells have membrane  $\alpha$ -chains. First, the



proportions of pronase-stripped and cultured cells bearing membrane f,g  $\alpha$ -chain determinants (i.e.,  $\alpha+$ ) are very similar to the proportions of  $\mu-,b+$  cells. Second, endogenous  $\mu$ -chains and  $\alpha$ -chains are not found on the same cells. Finally, all  $\alpha+$  cells double stain for b allotype determinants and hence have light chains (P. Jones, unpublished results). Thus all  $\alpha+$  cells are  $\mu-,b+$ . Staining analyses of appendix cells corroborated these findings.

It is significant that in all rabbits tested the proportions of total and of light chain-bearing lymphocytes which were also  $\alpha$ -bearing in the GALT (Peyer's patches and appendix) were higher than in the spleen, lymph node, and peripheral blood. The elevated proportions of IgA-bearing cells in the GALT compared to other lymphoid tissues correlate well with the precursor potentials of lymphocytes from these different cell sources. Both the Peyer's patches and the appendix are enriched sources of precursors for plasma cells synthesizing IgA compared to IgM or IgG. Other lymphoid tissues (i.e., lymph nodes and peripheral blood) give rise predominantly to IgG- and IgM-producing plasma cells. In addition, if the  $b+$  cells without either  $\mu-$  or  $\alpha$ -chains actually have membrane IgG, then their presence would be correlated with the relative abundance of precursors of IgG-producing plasma cells.

One common theme running through our studies of the synthetic potentials of rabbit B lymphocytes at varying stages of differentiation is that the majority of them appear to be restricted to the synthesis of one isotype and allotype of Ig. The terminally differentiated plasma cell secretes molecules of one class (20), type (21), allotype (22), and specificity (reviewed in 7). More recently we have shown that lymphocytes are already committed with respect to the allotype they and their progeny will produce by the time they display Ig molecules on their membranes (13, 16).

The current studies suggest that rabbit B lymphocytes may also be committed with respect to IgA and IgM heavy chain isotypes. Not only are individual lymphocytes restricted to synthesizing membrane Ig molecules with either  $\alpha-$  or  $\mu$ -chains, but isolated populations of  $\mu+$  and  $\mu-,b+$  (probably  $\alpha+$ ) Peyer's patch lymphocytes give rise to IgM- and IgA-producing plasma cells, respectively (11). However, we have little information concerning the commitment of rabbit lymphocytes with respect to the synthesis of IgG. After pronase stripping and overnight culture, no IgG-bearing cells were detectable by indirect immunofluorescence using a goat antirabbit-Fc $\gamma$  reagent.<sup>2</sup> Preliminary experiments with antibody reagents specific for the d11  $\gamma$ -chain allotype marker indicated that no more than a few percent of rabbit lymphocytes have membrane IgG, in agreement with results obtained by Pernis et al. (18).

Reports in the literature vary widely concerning both the relative proportions of lymphocytes bearing IgA, IgG, and IgM membrane molecules (18, 23, 24) and whether or not lymphocytes are restricted to expressing molecules of only one isotype on their membrane (18, 24-26). Two factors which may contribute to these discrepancies have been revealed during the study of rabbit membrane Ig. First, some antiheavy chain reagents react with determinants which are not exposed on the Ig molecules in lymphocyte membranes. Second, serum Ig molecules of different isotypes can bind to the membranes of rabbit lymphocytes.<sup>3</sup> Thus the superficial reactivities of antibody reagents with Ig

determinants on lymphocyte membranes may not always reflect the cells' true synthetic potential.

### Summary

Fluorescent antibody staining with antibodies to the *f* and *g* locus allotype markers present on rabbit  $\alpha$ -chains revealed that the  $\alpha$ -chain is the heavy chain on the Peyer's patch lymphocytes which previously had been shown to be the precursors of IgA-producing plasma cells. In addition, lymphocytes which had been stripped of membrane Ig with pronase and then cultured overnight to allow the sole expression of endogenous membrane Ig were found to have either the  $\mu$ -chain or the  $\alpha$ -chain on their membranes, but not both. These results suggest that most lymphocytes are restricted to the synthesis of one class of heavy chains at a time and that the commitment to synthesizing that particular heavy chain is maintained during the differentiation of lymphocytes into plasma cells.

The proportion of lymphocytes with membrane  $\alpha$ -chains is higher in the Peyer's patch and appendix, two gut-associated lymphoid tissues (GALT), than in other lymphoid tissues. Since the GALT are enriched sources of precursors for IgA-producing plasma cells compared to nongut-associated tissues, the presence of cells bearing membrane  $\alpha$ -chains correlates well with the relative abilities of these tissues to generate IgA plasma cells.

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