IgG SUBCLASS, IgE, AND IgA ANTI-TRINITROPHENYL ANTIBODY PRODUCTION WITHIN TRINITROPHENYL-FICOLL-RESPONSIVE B CELL CLONES

Evidence in Support of Three Distinct Switching Pathways

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Germline DNA contains a group of linked *Igh-C* genes that encode the constant regions of the distinct isotypic forms of immunoglobulin (Ig). How the expression of each *Igh-C* gene is regulated in antigen stimulated B lymphocytes is currently the topic of intense investigation in the fields of both molecular and cellular immunology.

Studies using myeloma cells have shown that the 5' to 3' *Igh-C* gene sequence on the 12th chromosome of the mouse is in the following order: 5' *Igh-μ*, *Igh-δ*, *Igh-γ3*, *Igh-γ1*, *Igh-γ2b*, *Igh-γ2a*, *Igh-ε*, and *Igh-α* 3' (1, 2). The secretion of each of these isotypes appears to require a genetic rearrangement, either at the DNA or RNA level, by which the *Igh-VDJ* gene complex, which codes for the variable region of the Ig molecule, is juxtaposed immediately 5' to the coding material for a given Ig heavy chain constant region (3, 4). A thorough understanding of the relationship of *Igh-C* gene order and *Igh-C* gene activation to the phenomenon of isotype switching within antigen-responsive B lymphocytes may provide approaches to the selective regulation of the production of the various Ig classes.

We have studied the production of antibodies (Ab)[1] of each Ig class within B cell clones stimulated by the type 2 antigen, trinitrophenyl (TNP)-Ficoll (5). This antigen stimulates members of a B cell subpopulation (Lyb-5$^+$ B cells) (6) and, in vivo, can do so in the absence of thymus-matured T lymphocytes, i.e., in athymic *nu/nu* mice (7).[2] Our results have indicated that, in the absence of added T cells, the amount of anti-TNP Ab of each IgG isotype produced in response to TNP-Ficoll directly correlates with the 5' to 3' *Igh-C* gene order (5, 7). Furthermore, results from the splenic focus assay revealed that the clonal frequency of precursors of anti-TNP Ab-secreting cells producing IgM and the IgG isotypes also directly correlated with this gene order (5). These observations, plus the finding that B cell clones that secreted a given subclass of IgG anti-TNP Ab tended to coexpress anti-TNP Ab of each of the isotypes encoded by 5' *Igh-C* genes (5), suggested that *Igh-C* gene order strongly influences the isotype switching events within B cell clones responding to TNP-Ficoll. We have further

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[1] *Abbreviations used in this paper:* Ab, antibody; BSA, bovine serum albumin; C, complement; PC, phosphorylcholine; RIA, radioimmunoassay; S, switch; TNP, trinitrophenyl.

[2] In in vitro cell suspension culture, Thy-1$^+$, Lyt-1$^+$ cells have been shown to be necessary for Ab responses to TNP-Ficoll (8).

shown that T lymphocytes appear to enhance the IgG switching mechanism within TNP-Ficoll-responsive B cell clones. The result of such an enhancement is a preferential increase in expression of those IgG subclasses, namely IgG2a and IgG2b, which are encoded by genes at the 3' end of the *Igh-γ* gene complex and which have the smallest probability of being expressed by B cells which have been depleted of T cells (5, 7).

In this report, we have extended our study of TNP-Ficoll-induced isotype expression to the two additional isotypes which are encoded by genes 3' to the *Igh-γ* gene cluster, i.e., IgE and IgA. The results suggest that switches to the IgG subclasses, to IgE, and to IgA are, at least in part, mediated by three distinct pathways.

Materials and Methods

Animals. C57BL/10 ScN *nu/nu* C57BL/10 ScN *nu/* +, and C57BL/10 ScN +/+ mice were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health. Mice were used at 2–3 mo of age.

Antigens. TNP-aminoethylcarbamylmethyl-Ficoll (TNP-Ficoll), described in preceding reports (5, 7), was used to stimulate anti-TNP Ab for isotype analysis. TNP-bovine serum albumin (TNP-BSA; 15 mol TNP/mol BSA) was prepared as described previously (7).

Preparation of B and T Cell-enriched Populations. B cells were purified for use in the splenic focus assay by methods described previously (5, 7). Briefly, *nu/* + spleen cell suspensions were depleted of Thy-1.2-positive cells and Lyt-1-positive cells by treatment with specific monoclonal antibodies and complement (C). T cells were selectively enriched from *nu/* + spleen cells by the plate-binding method described by Mage et al. (9) and used previously by this laboratory (5, 7). T cells used in the splenic focus assay were in addition treated with 17C9 (10), a monoclonal anti-ThB antibody (11), and C, as described previously (5).

Radioimmunoassay (RIA) for Measurement of Anti-TNP Antibody. The solid-phase RIA described previously (7), which uses TNP-BSA-coated flexible microtiter plates and ^{3}H-labeled anti-isotype reagents, was used to measure anti-TNP Ab of the various classes in immune serum and in splenic focus assay culture supernatants.

The preparation and affinity-purification of goat anti-IgM, goat anti-IgG1, goat anti-IgG2a, goat anti-IgG2b, rabbit anti-IgG3, and goat anti-IgA has been previously described (7, 12). Affinity-purified rabbit anti-IgE was the kind gift of Dr. David Katz, Medical Biology Institute, La Jolla, CA. These antibodies were tritiated by reductive formylation with NaB^{3}H$_4$ (13). With the exception of the anti-IgE Ab, these reagents have been previously shown to have comparable efficiencies for binding to their respective insolubilized Ig class substrates (7, 12). To analyze the binding efficiency of the ^{3}H-labeled anti-IgE reagent, we have compared the binding of [^{3}H]anti-IgE, [^{3}H]anti-IgA, [^{3}H]anti-IgG1, and [^{3}H]anti-IgG2b to purified anti-TNP hybridoma or myeloma antibodies of the appropriate isotype in a TNP-specific RIA. The data in Fig. 1 show that similar binding curves are generated when these labeled reagents are used to measure various concentrations of the appropriate anti-TNP antibody. These data and data presented elsewhere (7, 12) indicate that our isotype-specific RIA is capable of detecting approximately equivalent concentrations of anti-TNP Ab of each isotype.

Splenic Focus Assay for Enumeration of Anti-TNP Ficoll-responsive B Cells. The modification of the splenic focus system used to analyze TNP-Ficoll specific B cells has been previously described (5). Briefly, limiting numbers of unprimed B cells with or without 5 × 10^6 unprimed T cells were transferred intravenously into 1,600-rad ^{137}Cs-irradiated unprimed syngeneic *nu/nu* recipient mice. Approximately 15 h after cell transfer, the recipients were injected intravenously with 10 μg TNP-Ficoll; 1 h later, the mice were killed. The recipient spleens were sliced into 1-mm^3 fragments, which were then prepared as individual organ cultures. Culture supernatants were changed every 3–4 d. Supernatants collected from days 10 through 17 of culture were tested for anti-TNP Ab of the various isotypes by the solid-phase RIA described above. To determine the percent of anti-isotype bound that would be used to categorize supernatants as positive or negative, we constructed a histogram of percent of fragments from an individual spleen that bound given percents of anti-isotype. When percent of fragments was plotted
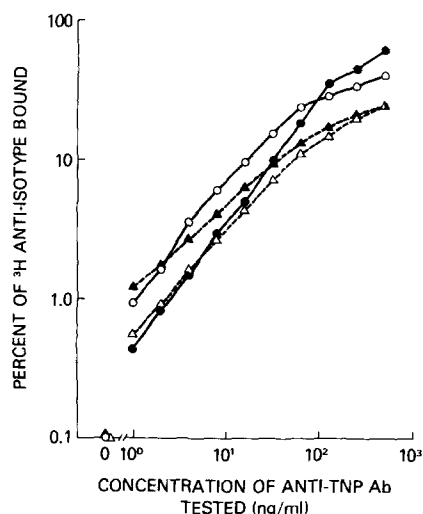
FIG. 1.   Analysis of binding of ³H-labeled anti-isotype reagents to monoclonal anti-TNP antibodies (IgG1 hybridoma [O], IgG2b hybridoma [●], IgE hybridoma [△], IgA myeloma [MOPC-315] [▲]). Various concentrations of purified monoclonal proteins were bound to TNP-BSA coated microtiter wells. ³H-labeled affinity-purified isotype-specific antibodies were added at 30,000 cpm/ 50 μl/well. The percent of added ³H-labeled affinity-purified antibody bound was subsequently determined.

against percent anti-isotype bound, a bimodal histogram was obtained. We utilized the "trough" in this histogram as the "cut-off" between positive and negative fragments. Through this type of analysis, occasional fragments from spleens containing B cells but no antigen were classified as positive. However, supernatants from these "positive" fragments contained considerably less anti-TNP antibody than supernatants from most positive fragments obtained from spleens containing B cells plus antigen.

Results

Serum Analysis of IgM, IgG3, IgG1, IgG2b, IgG2a, IgE, and IgA Anti-TNP Ab Responses to TNP-Ficoll in nu/nu and T Cell-reconstituted nu/nu Mice.   Our previous studies have shown that the amounts of IgM anti-TNP Ab and anti-TNP Ab of each IgG isotype produced in response to TNP-Ficoll in the absence of T cells directly correlate with the 5′ to 3′ *Igh-C* gene order (5, 7). Thus, the isotypic hierarchy characteristically observed is IgM > IgG3 > IgG1 > IgG2b > IgG2a. We wished to determine whether the production of Ab coded for by the most 3′-encoded *Igh-C* genes, i.e., IgE and IgA, would also fit this correlation with the 5′ to 3′ *Igh-C* gene order. Furthermore, we wanted to determine whether the production of IgE and IgA Ab might be influenced strongly by the presence of T lymphocytes, like the production of the 3′-encoded IgG isotype, IgG2a (5, 7).

The data in Table I show results from three experiments in which the anti-TNP Ab responses of *nu/nu* and T cell-reconstituted *nu/nu* mice to TNP-Ficoll were measured. No IgE anti-TNP Ab above control levels could be detected in sera from either *nu/nu* or T cell-reconstituted *nu/nu* mice. All groups of mice produced IgA anti-TNP Ab to TNP-Ficoll. The concentrations of serum IgA Ab were less than that of IgG2a, the IgG subclass with the lowest serum anti-TNP concentration. Mice reconstituted with T cells had higher serum IgA anti-TNP titers than nonreconstituted *nu/nu* mice, but

TABLE I

*Isotypic Analysis of Serum Anti-TNP Ab Titers in TNP-Ficoll-immunized nu/nu and
T Cell-reconstituted nu/nu Mice*

Experiment	Mice tested	Reciprocal of peak anti-TNP serum titer						
		IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
1	nu/nu	51,200	16,400	5,120	200	40	—*	40
	nu/nu + T	30,000	12,000	10,240	800	2,000	—	120
2	nu/nu	—	—	13,000	—	1,280	—	150
	nu/nu + T	—	—	60,000	—	10,240	—	1,500
3	nu/nu	55,000	35,000	25,600	—	2,200	<5	116
	nu/nu + T	150,000	55,000	80,000	—	24,000	<5	790

Anti-TNP Ab was measured in sera obtained on days 5, 7, 10, 14, and 20 after immunization of mice with 10 μg TNP-Ficoll in saline. A pool of equal aliquots of sera within each group (n = 4–5) was tested from each day. Data shown are the peak anti-TNP titers in each isotype. Titer is defined as the serum dilution at which 1% of the added [^{3}H]anti-isotype counts are bound. Reciprocal anti-TNP titers in unimmunized nu/nu mice were, on the average, 3,600 for IgM and <10 for IgG3, IgG1, IgG2b, IgG2a, IgE, and IgA. T cell reconstituted nu/nu mice received 5 × 10^{6}–10^{7} C57BL/10 T cells 1 d before antigen.
* Not tested.

the T cell enhancement was generally not as substantial as that seen in the IgG2a Ab response. Because comparable results were observed when production of anti-TNP Ab of the various isotypes from immune cells was measured with the use of an in vitro short-term secretion assay (12) (data not shown), the relatively low IgE and IgA anti-TNP serum titers seen when immune sera were analyzed cannot be attributed solely to the more rapid in vivo clearance of these Ig (14, 15).

Analysis of the Frequency of TNP-Ficoll Responsive B Cell Clones that Produce Various Isotypes in the Splenic Focus Assay. Our recent studies of the IgM and IgG isotypic responses to TNP-Ficoll demonstrated that the relative frequency of B cell clones that produce anti-TNP Ab of each of the IgG isotypes in the splenic focus assay correlated with the relative titers of anti-TNP Ab of these isotypes observed in the sera of immune mice (5). We wished to determine whether the frequency of B cell clones that produced IgA and IgE anti-TNP Ab to TNP-Ficoll would follow a similar pattern and thus correspond to the relatively low serum levels of anti-TNP Ab of these isotypes produced in vivo. The data shown in Table II indicate that this is not the case. Of all the fragments prepared from donors that received B cells only, none were found to secrete IgG2a anti-TNP Ab. However, a considerable number were found to secrete IgE or IgA anti-TNP Ab. In fact, the frequency of both IgE- and IgA-positive fragments was found to be higher than the frequency of IgG1-positive fragments. This is in clear contradistinction to the relative serum titers of anti-TNP Ab of the various isotypes in the experiments reported in Table I. Both the in vivo response, as measured by serum analysis, and the in vitro splenic focus assay response show that, in the absence of T cells, the relative production of anti-TNP Ab of the various IgG subclasses correlates with 5' to 3' *Igh-C* gene order. However, the splenic focus assay response shows that the frequency of IgE and IgA anti-TNP-secreting clones does not directly correlate with this gene order.

When the frequency of isotype expression was compared in fragments containing B cells only vs. those containing B cells and T cells, it was found that the presence of T cells significantly enhanced the frequency of fragments secreting IgA anti-TNP Ab

TABLE II

Analysis of the Frequency of Clones Secreting Anti-TNP Ab of Various Isotypes in Fragments Containing B Cells Only or B Cells Plus T Cells*

Cells trans- ferred	TNP- Ficoll	Total num- ber frag- ments stud- ied	Total number B cells injected	Number of fragments secreting anti-TNP Ab per 10^6 B cells injected (X ± SE)						
				IgM	IgG3‡	IgG1	IgG2b	IgG2a‡	IgE	IgA
			× 10^6							
B	—	124	5.75	0.67 ±0.48	0.4	0.13 ±0.13	0.17 ±0.17	0	0	0
B + T	—	178	9.00	0.65 ±0.34	0.4 ±0.4	0.05 ±0.05	0	0	0	0
B	+	723	34.25	3.63 ±0.42	1.85 ±0.34	0.41 ±0.10	0.35 ±0.10	0	0.74 ±0.13	1.49 ±0.26
B + T	+	611	26.75	4.48 ±0.46	2.56 ±0.63	0.65 ±0.20	1.01 ±0.23	0.48 ±0.20	0.94 ±0.22	2.74 ±0.37
P value				NS§	NS	NS	<0.01	<0.02	NS	<0.01

* Irradiated recipients which received T cells only plus TNP-Ficoll had 0.07 ± 0.07 IgM-positive fragments, 0.07 ± 0.07 IgG1-positive fragments, 0.07 ± 0.07 IgE-positive fragments, and no IgG2b- or IgA-positive fragments per 10^6 T cells injected. The mean and SE values are calculated from the number of positive fragments from individual recipient spleens within a group.

‡ The data for IgG3 and IgG2a positive fragments in the above groups were available from only one of the three pooled experiments. This experiment had the following dimensions (total number of fragments studied, total number of B cells injected): B cells, no antigen (36 fragments, 2.5 × 10^6 B cells injected); B + T cells, no antigen (82 fragments, 5 × 10^6 B cells injected); B cells + antigen (334 fragments, 20 × 10^6 B cells injected); and B + T cells + antigen (210 fragments, 12.5 × 10^6 B cells injected).

§ Not significant. P values are derived by comparing results from TNP-Ficoll-immunized groups receiving B cells and B cells plus T cells.

but had no significant effect on the frequency of fragments secreting IgE anti-TNP Ab. As described previously (5), the frequency of fragments secreting IgG2a and IgG2b anti-TNP Ab was significantly enhanced by the presence of T cells.

Analysis of the Concentration of Anti-TNP Ab of Each Isotype Found in Supernatants of Positive Fragments. It is conceivable that the divergent observations on relative IgG, IgE, and IgA anti-TNP Ab production obtained through serum and clonal analysis could be explained by a lower production of IgA and IgE anti-TNP Ab than IgG anti-TNP Ab in individual clones. We therefore compared the average concentration of IgG1, IgG2b, IgE, and IgA anti-TNP Ab in positive clones. The data in Table III show that the average production of IgE anti-TNP Ab per fragment was lower than the average production of IgG1 and IgG2b anti-TNP Ab. Thus, a lower relative production of IgE may contribute to the failure to detect substantial serum titers of IgE anti-TNP Ab in the serum of immune mice. However, because the differences between IgE and the IgG subclasses in average anti-TNP Ab production within clones are not as great as the differences in serum responses between these isotypes, it is unlikely that this is the sole explanation. In addition, the data in Table III show that the amount of IgA anti-TNP Ab produced by individual clones was considerably higher than the production of anti-TNP Ab of either the IgG1 or IgG2b subclasses. Thus, with regard to synthesis of IgA anti-TNP Ab, our combined results clearly show

TABLE III

Analysis of the Concentration of Anti-TNP Ab of Each Isotype Found in Supernatants of Positive Fragments

Fragments containing		IgG1	IgG2b	IgE	IgA
B cells	Mean	2.5	10.5	1.3	19.3
	Range	0.6–31	2.5–100	0.5–6	1.5–500
B + T cells	Mean	5.8	7.7	1.5	33.7
	Range	0.5–70	1–250	0.5–4	1.5–350

* Ab in supernatants from positive fragments was quantitated through the use of standard curves obtained with purified IgG1, IgG2b, and IgE anti-DNP hybridoma proteins, and purified MOPC-315 IgA anti-TNP myeloma protein.

TABLE IV

*Analysis of the Coexpression of Anti-TNP Antibody of Additional Isotypes in Fragments Secreting a Given Isotype of Anti-TNP Antibody**

Cells trans-ferred	Fragments se-creting the fol-lowing isotype of anti-TNP Ab	n	Percent of fragments secreting anti-TNP Ab of the following number of isotypes							X number of isotypes per frag-ment
			1	2	3	4	5	6	7	
B	IgM	68	26.5	45.6	13.2	10.3	2.9	1.5	0	2.22
	IgG3	37	0	56.8	18.9	16.2	5.4	2.7	0	2.78
	IgG1	11	0	27.3	0	45.5	18.2	9.1	0	3.82
	IgG2b	10	20.0	10.0	0	40.0	20.0	10.0	0	3.60
	IgG2a	0	—	—	—	—	—	—	—	—
	IgE	13	38.5	23.1	23.1	7.7	0	7.7	0	2.31
	IgA	29	27.6	17.2	27.6	17.2	6.9	3.4	0	2.69
B + T	IgM	38	18.4	34.2	10.5	21.0	10.5	5.3	0	2.87
	IgG3	32	6.3	40.6	18.8	21.9	12.5	0	0	2.94
	IgG1	8	0	0	0	37.5	37.5	25.0	0	4.31
	IgG2b	16	6.3	12.5	6.3	43.8	18.8	12.5	0	3.94
	IgG2a	6	0	0	16.7	16.7	50.0	16.7	0	4.67
	IgE	5	20.0	20.0	20.0	0	20.0	20.0	0	3.40
	IgA	41	51.2	9.8	12.2	17.1	4.9	4.9	0	2.30

* Total fragments studied in the B cell only group = 334; total fragments studied in the B + T group = 210.

that the low IgA/IgG ratios observed when serum anti-TNP Ab titers are analyzed cannot be explained by either a diminished frequency of IgA-positive anti-TNP clones or by lower production of IgA anti-TNP Ab within positive clones.

Appearance of Anti-TNP Ab of Several Isotypes in Individual TNP-Ficoll-responsive B Cell Clones. Splenic fragments that appear to have received a single antigen-responsive precursor have been shown to produce Ab of multiple isotypes (5, 16–19). This has been taken as strong evidence for intraclonal isotype switching. Our previous studies (5) have shown that clones that produce IgG2a anti-TNP Ab in response to TNP-Ficoll tend to be actively switching clones, i.e., they produce not only IgG2a but many other isotypes. In Table IV we present an analysis of the complexity of isotype expression in the anti-TNP Ab produced by TNP-Ficoll responsive splenic fragments, including data on the secretion of IgE and IgA. Fragments secreting anti-TNP Ab were categorized as those producing Ab of the IgM, IgG3, IgG1, IgG2b, IgG2a, IgE, or IgA isotypes. The percent of these fragments which secreted anti-TNP Ab of only

the designated isotype or of one to six additional isotypes was then evaluated. The data indicate that fragments which secreted IgE or IgA anti-TNP Ab appeared to coexpress anti-TNP Ab of fewer additional isotypes than did fragments secreting IgG1, IgG2b, or IgG2a. Among clones producing a given class, the frequency of clones that secreted anti-TNP of only that class was very low for IgG subclasses (4%), but considerably higher for IgM (24%), IgE (33%), and IgA (41%). Finally, the data shown are consistent with our previous studies (5), which demonstrated that the presence of T cells generally tends to increase the number of different isotypes of anti-TNP Ab synthesized by TNP-Ficoll-responsive B cell clones. An exception to this was found in the case of IgA positive clones, in which T cells appeared to increase the proportion of clones that secreted only IgA anti-TNP Ab. Thus, the increase in frequency of IgA-positive clones in the presence of T lymphocytes (Table II) appears to be primarily due to an increase in the frequency of clones which secrete only IgA anti-TNP Ab.

We have previously found (5) that TNP-Ficoll-stimulated clones, which produce a given IgG subclass of anti-TNP Ab, have a high probability of also producing IgM anti-TNP Ab and anti-TNP-Ab of each IgG subclass whose *Igh-C* gene is encoded 5′ to the gene for the given subclass. The data in Fig. 2 and Table V show that clones which produce IgE and IgA anti-TNP Ab do not exhibit such a high probability of coexpression of 5′ encoded isotypes. The data in Fig. 2 were taken from an experiment in which TNP-Ficoll-stimulated fragments containing B and T cells were analyzed for the production of anti-TNP Ab of all isotypes with the exception of IgD. The percent of fragments which secreted anti-TNP Ab of a designated isotype (column A) that coexpressed anti-TNP Ab of each additional isotype (column B) is indicated. The majority of fragments that secreted a designated IgG anti-TNP Ab also secreted all, or nearly all, the 5′-encoded isotypes. By contrast, only a small proportion of the fragments that secreted IgE or IgA anti-TNP Ab also secreted anti-TNP Ab of any given 5′-encoded IgG isotype. Comparable data were obtained when fragments containing B cells only were analyzed, with the exceptions that no clones secreting IgG2a anti-TNP Ab were detected, and that a higher proportion (69%) of the IgA anti-TNP Ab-producing fragments also produced IgM anti-TNP Ab (data not shown). When data were pooled from three experiments in which production of B and T cell-containing fragments were analyzed for production of IgM and IgA anti-TNP Ab, an average of 51% of the IgA producing fragments were also found positive for IgM—a slightly higher percentage than that observed in the experiment in Fig. 2.

Teale et al. (18, 20) have suggested that a subset of surface IgM-negative, immature B cells can generate clones that secrete IgA Ab without IgG Ab. In our experiments, the reasonably large proportion of clones that secrete only IgA anti-TNP Ab may be derived from such progenitors. Alternatively, these clones may have arisen from mature B cells that had previously switched to IgA. We wished to exclude these populations of cells from our analysis and to ascertain whether IgA-producing clones, which coexpress IgM and thus probably originate from IgM-positive precursors, exhibit a high frequency of coexpression of all the 5′-encoded isotypes. To do so, we have analyzed the coexpression of anti-TNP Ab of various isotypes in clones positive for IgM as well as IgA anti-TNP Ab. We found the proportion of clones secreting IgM and IgA anti-TNP Ab that also coexpressed IgG3, IgG1, IgG2b, IgG2a, or IgE to be 87%, 26%, 38%, 13%, and 21%, respectively. With the exception of IgG3, these
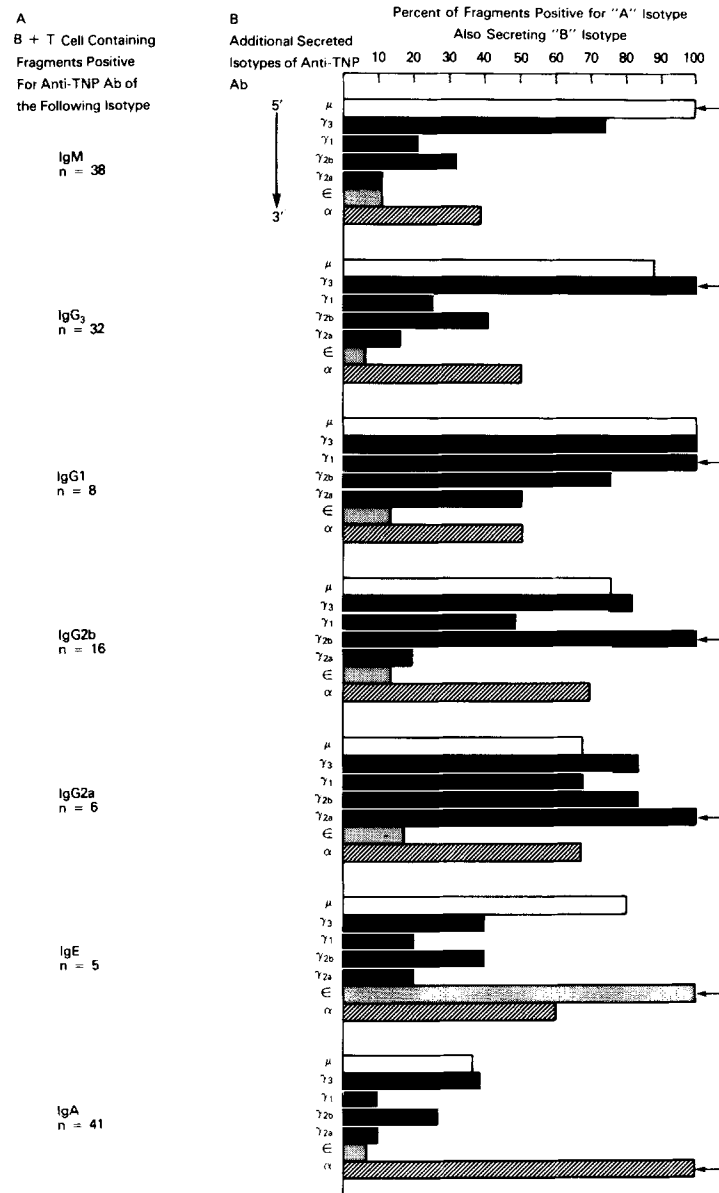
FIG. 2. Analysis of anti-TNP Ab isotype coexpression in TNP-Ficoll stimulated fragments containing B + T cells.

proportions are relatively similar to the proportion of total IgA positive anti-TNP clones which also secreted the indicated isotypes. Thus, clones producing IgA anti-TNP Ab do not have a high propensity for coexpression of anti-TNP Ab of most of the 5'-encoded IgG and IgE isotypes. This is true whether total IgA-positive anti-TNP clones or clones secreting both IgA and IgM anti-TNP Ab are analyzed.

An analysis of the frequency of coexpression of anti-TNP Ab of all 5'-encoded

TABLE V

Association of Expression of a Given Isotype of Ab in TNP-Ficoll Stimulated Fragments with Coexpression of Ab of Isotypes Encoded by 5' or 3' Igh-C Genes

Cells within fragments	Designated isotype	Frequency of total fragments positive for desig- nated isotype (positive/total tested)	Frequency of coexpression of all 5'-encoded isotypes	Frequency of coexpression of all 3'-encoded isotypes
B cells	IgM	68/334	—	0/68 (0%)
	IgG3	37/334	37/37 (100%)	0/37 (0%)
	IgG1	11/334	7/11 (64%)	0/11 (0%)
	IgG2b	10/334	5/10 (50%)	0/10 (0%)
	IgG2a	0/334	0/0 (—)	0/0 (—)
	IgE	13/334	0/13 (0%)	4/13 (31%)
	IgA	29/334	0/29 (0%)	—
B + T cells	IgM	38/210	—	0/38 (0%)
	IgG3	32/210	28/32 (88%)	0/32 (0%)
	IgG1	8/210	8/8 (100%)	0/8 (0%)
	IgG2b	16/210	6/16 (38%)	0/16 (0%)
	IgG2a	6/210	3/6 (50%)	0/6 (0%)
	IgE	5/210	0/5 (0%)	3/5 (60%)
	IgA	41/210	0/41 (0%)	—

TABLE VI

Coexpression of Anti-TNP Antibody of Different Isotypes in Splenic Fragments *

Isotypes	Number of positive fragments‡			χ^2 (P) for coexpression	
	+, +	+, −	−, +	All fragments (n = 1334)	Ab-producing fragments (n = 318)
IgG1 + IgG2b	18	16	24	284 (<0.001)	52 (<0.001)
IgG1 + IgE	5	29	40	14 (<0.001)	0.01 (NS)
IgG2b + IgE	6	36	39	16 (<0.001)	0.01 (NS)
IgA + IgE	16	113	29	36 (<0.001)	0.6 (NS)
IgG1 + IgA	23	11	106	134 (<0.001)	12 (<0.001)
IgG2b + IgA	26	16	103	136 (<0.001)	9.1 (<0.005)

* Data from three separate experiments involving fragments from donors receiving B cells only or B cells + T cells were pooled.

‡ +, + denotes fragments positive for both members of the isotypic pair; +, − denotes fragments positive for the first and negative for the second member of the isotypic pair; −, + denotes fragments negative for the first and positive for the second member of the isotypic pair.

isotypes in fragments secreting anti-TNP Ab of a given isotype is shown in Table V. This analysis clearly shows that, whereas a considerable proportion of clones secreting a given IgG subclass also secrete all the 5'-encoded isotypes, none secrete all the 3'-encoded isotypes. Furthermore, unlike these IgG-positive fragments, none of the IgE or IgA positive fragments were found to coexpress all the 5'-encoded isotypes.

We have performed a statistical comparison (Table VI) of the frequency of coexpression of IgG1, IgG2a, IgE, and IgA anti-TNP Ab using pooled data from three separate experiments. The χ^2 and probability values for coexpression were determined from the frequency of fragments positive for anti-TNP Ab of two individual isotypes

among all fragments or among those fragments that were positive for anti-TNP Ab of any isotype. The analysis of coexpression of each pair of isotypes based on their frequency among all fragments shows that the presence of any two isotypes of anti-TNP Ab within a single fragment is highly unlikely to be caused by chance overlap of two independent progenitors. The very significant χ^2 values suggest, rather, that the coexpression is caused by multiple switching events occurring within daughter cells of a common single precursor. The purpose of analyzing the coexpression of each pair of isotypes of anti-TNP Ab among all anti-TNP Ab-secreting fragments was to determine whether the coexpression of two given isotypes within a responsive clone can be accounted for by independent, random events or whether the factors that lead to the expression of one class of anti-TNP Ab make it more or less likely that the other class will be expressed in that clone. The results indicate that the switch to production of IgG2b anti-TNP Ab has a very high probability of being accompanied by a switch to production of IgG1 anti-TNP Ab within the same clone ($\chi^2 = 52$). Clones that produce IgE anti-TNP Ab, however, express anti-TNP Ab of IgG1, IgG2b, or IgA isotypes at essentially the same frequencies that these Ab are produced among all fragments producing anti-TNP Ab. Thus, it appears that the switch to IgE anti-TNP Ab production is independent of intraclonal switches to IgG or IgA in clones responding to TNP-Ficoll. Clones that produce IgG1 or IgG2b anti-TNP Ab do have a significantly greater likelihood of producing IgA anti-TNP Ab than would be expected by chance alone, although the degree of association is not as great as that for IgG1 and IgG2b ($\chi^2 = 12$ and 9.1 for IgG1-IgA and IgG2b-IgA, respectively).

Discussion

Our previous analysis (5, 7) of the IgG subclass of anti-TNP antibodies produced in response to TNP-Ficoll has suggested that the 5' to 3' genomic order of the various *Igh-γ* genes influences the frequency of switching to or expression of these isotypes. Thus, the results of studies analyzing the serum anti-TNP Ab levels in immunized *nu/nu* mice, the in vitro secretion of anti-TNP Ab from spleen cells of immunized mice, and the frequency of clones producing anti-TNP Ab of each IgG subclass in the splenic focus assay all demonstrate that the isotypic production hierarchy correlates directly with 5' to 3' *Igh-C* gene order: IgM > IgG3 > IgG1 > IgG2b > IgG2a.

Because of this strong association of the IgG subclass response with *Igh-γ* gene order, we wished to determine how the gene position of the most 3'-encoded isotypes, i.e., IgE and IgA (1, 2), affected their representation in the anti-TNP Ab response to TNP-Ficoll. In an attempt to study the isotype switch to IgE and IgA, we have analyzed anti-TNP Ab titers in serum from TNP-Ficoll-immunized mice and the frequency of antigen-responsive B cell clones in the splenic focus assay. In this report we have shown that the results obtained from these two approaches differed substantially. Although the serum analysis revealed low titers of IgE and IgA anti-TNP Ab in response to TNP-Ficoll, results from the splenic focus assay indicated that a relatively large proportion of TNP-Ficoll responsive B cell clones produce IgE or IgA anti-TNP Ab. We believe the focus assay is the more reliable approach for a study of isotype switching because it allows the analysis of switching patterns within clones arising from individual antigen-responsive B lymphocytes. Possible reasons for the apparent discrepancy between data from this in vitro system and in vivo data will be considered at the end of this discussion.

Results from the splenic focus assay show that TNP-Ficoll responsive fragments produce a number of isotypes of anti-TNP Ab. The clonal nature of the focus assay strongly implies that this is due to intraclonal isotype switching events (16–19). Because IgM anti-TNP Ab was found in the majority of clones secreting IgG or IgE anti-TNP Ab and in approximately one-half of the IgA anti-TNP Ab secreting clones, it is likely that most clones secreting multiple isotypes of anti-TNP Ab originated from IgM-positive precursors.

Hurwitz et al. (21) have recently reported that TNP-Ficoll-stimulated clones make IgM and IgA anti-TNP Ab but no IgG anti-TNP Ab in the splenic focus assay. This variation between their results and ours may be due to any of several differences in the culture and assay systems used: (a) In our splenic focus assay, TNP-Ficoll was injected intravenously 1 h before mice were killed and their spleens fragmented for in vitro culture; in the splenic focus assay used by Hurwitz et al., TNP-Ficoll was added directly to the in vitro culture. (b) We have used lethally irradiated unprimed nu/nu athymic recipients in the splenic focus assay, whereas Hurwitz et al. have used lethally irradiated unprimed +/+ euthymic recipients. (c) The ^{3}H RIA, which we used to detect anti-TNP Ab in splenic focus assay supernatants, was capable of detecting anti-TNP Ab at ~0.2 ng/ml with counts twice above background. This is considerably more sensitive than the ^{125}I RIA described in the above report. It should be noted that we have generally found IgG anti-TNP Ab to be in lower concentration than IgM or IgA anti-TNP Ab in splenic focus assay clones. Thus, a decrease in assay sensitivity could preclude the identification of many IgG-positive clones.

Our results suggest that three distinct switching pathways may be used during the expansion of TNP-Ficoll responsive B cell precursors. Whereas switches to each of the IgG subclass genes probably depend upon a common switching mechanism, the switch to IgE and the switch to IgA appear to occur independently and through distinct pathways. A common pathway for Igh-γ gene switching is suggested by the high degree of 5'-encoded isotype coexpression within the group of IgG subclasses. In this pathway, the major controlling factor for isotype expression appears to be Igh-γ gene order. IgE production and IgA production, on the other hand, appear to be mediated by distinct switching pathways. Thus, we have found that, unlike the IgG subclasses, the chromosomal order of the Igh-ε gene and the Igh-α gene does not influence the relative frequency of clones producing IgE or IgA anti-TNP Ab in response to TNP-Ficoll. Even in the absence of T cells, the frequency of IgE- or IgA-positive clones was found to be higher than the frequency of clones expressing most of the IgG isotypes. Furthermore, again unlike the IgG isotypes, clones that produce IgE or IgA do not exhibit a high degree of 5'-encoded gene coexpression.

Whereas, statistically, the association of IgG, IgA, and IgE anti-TNP Ab synthesis was found to be less significant than the associated expression of the various IgG subclasses, IgG, IgE, and IgA were occasionally found synthesized together in the same clone. This is an important observation as it suggests that IgG, IgA, and IgE switching events are not mutually exclusive. This observation supports the results of Teale et al. (19), which indicated that B cells producing IgG, IgA, and IgE are not necessarily derived from separate precursors which are each precommitted to the synthesis of a given immunoglobulin class.

The schematic in Fig. 3 summarizes our interpretation of the data presented in this report. Upon stimulation by the antigen, TNP-Ficoll, an IgM-positive precursor B cell, will proliferate. Its daughter cells will then enter one of the three main switching

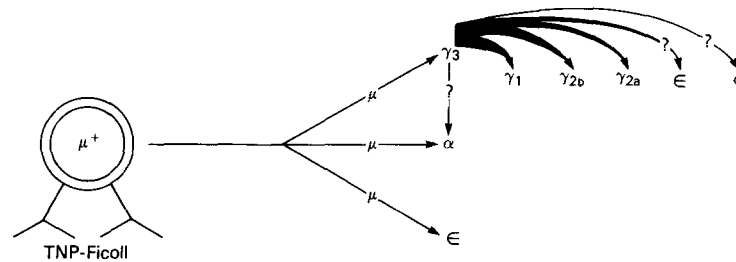Proposed Switching Pathways in Responses to TNP-Ficoll

FIG. 3.   Hypothetical model for the switching pathways utilized by a B cell clone responding to the antigen TNP-Ficoll.

pathways, i.e., to the synthesis of IgG subclasses, to IgE synthesis, or to IgA synthesis. Within the IgG switching pathway, a switch from *Igh-μ* to *Igh-γ3* may precede switches to the other *Igh-γ* genes. This possibility is favored (*a*) by the fact that most clones that secreted IgG1, IgG2b, or IgG2a also secreted IgG3 and (*b*) by recent molecular studies indicating that the switch (S) region 5' to the *Igh-μ* gene, i.e., Sμ, has more homology with Sγ3 than with other Sγ regions (1) and, furthermore, that Sγ3 has greater homology with the switching regions of the other *Igh-γ* genes than does Sμ.[3]

It should be noted that our results cannot distinguish between whether switches from *Igh-γ3* to the other *Igh-γ* genes occur as sequential switching events within one lineage of daughter cells, i.e., IgG3 → IgG1 → IgG2b → IgG2a, or whether distinct IgG3 → IgG1, IgG3 → IgG2b, and IgG3 → IgG2a switches occur within separate daughter cells. The fact that a very high proportion of IgG2a-positive clones also secrete IgG2b and IgG1 Ab does not necessarily favor the former possibility. Distinct probabilities may exist for each of the IgG3 → IgG1, IgG3 → IgG2b, and IgG3 → IgG2a events. Because *Igh-γ2a* is the most distal *Igh-γ* gene, switches to IgG2a may have the lowest probability, and clones containing cells that have undergone this event may thus have a high likelihood of also containing cells which have undergone the IgG3 → IgG1 and the IgG3 → IgG2b switches.

Although our data support the existence of distinct IgA, IgE, and IgG switching pathways, it is possible that occasional switches to IgE or to IgA may occur within a lineage of daughter cells that have entered the IgG switching pathway. Indeed, Gearhart et al. (17) have evidence that supports both a direct IgM → IgA switch and a IgM → IgG → IgA switch within separate daughter cells of a B cell clone. Our data do not eliminate IgM → IgG → IgE, IgM → IgG → IgA, or IgM → IgG → IgE → IgA switching pathways, but they strongly suggest that the majority of switches to IgA and to IgE, in the B cell response to TNP-Ficoll are not dependent upon prior IgM → IgG switching events.

87% of clones secreting both IgM and IgA anti-TNP Ab were found to coexpress IgG3 anti-TNP Ab whereas many fewer coexpress other isotypes. This high degree of IgG3 coexpression could be simply due to clones that have concurrently entered the

[3] L. W. Stanton and K. B. Marcu. Nucleotide sequence and properties of the murine γ[3] Ig heavy chain gene switch region: implications for successive Cγ gene switching. Manuscript submitted for publication.

IgA and IgG switching pathways; because IgG3 is the most likely switch within the IgG pathway, it would be the most highly represented. Alternatively, as illustrated in Fig. 3, this high degree of IgG3 coexpression could partly be caused by an IgM → IgG3 → IgA shunt. This latter possibility is given some support by Stanton and Marcu's finding that Sγ_3 and Sα possess the highest concentration of a particular switch sequence, YAGGTTG, when compared with Sμ, Sγ_1, and Sγ_{2b}.[3]

The concept of separate IgA, IgE, and IgG switching pathways is supported by a number of molecular and cellular observations. Thus, molecular analyses have revealed an overall greater homology of Sα with Sμ than with any of the IgG switch regions (1),[3] and the existence of IgA-specific switching sites that may use class-specific switching enzymes has been proposed (22). The observation by Teale et al. (18, 20) that neonatal B cells appear to acquire the ability to produce IgA before IgG (18, 20) is compatible with a distinct IgA switching pathway. In addition, Kuritani and Cooper (23) have recently presented evidence that human B cell differentiation from IgM production to IgG or to IgA production is largely mediated by independent switching events. A distinct IgE switching pathway has been previously suggested by the preferential induction of IgE synthesis and IgE helper factors after certain stimuli (24-27), by the differential sensitivity of IgG and IgE B cells to certain immuno-modulatory regimens (28-31), and by the capacity of mice with xid-determined immune defect to produce large amounts of anti-phosphorylcholine (PC) Ab of the IgE class but to synthesize poorly anti-PC Ab of IgG and IgM classes (32). An exception to this is a recent report suggesting linkage of IgE and IgA expression in certain rat B cell populations (33).

It should be emphasized that although our results suggest that intraclonal switches to IgA, IgE, and IgG subclasses may be mediated by distinct pathways, perhaps controlled by distinct switching enzymes, they clearly do not indicate that IgM-positive B cell precursors are precommitted to one pathway or another. As mentioned previously, IgG, IgA, and IgE switching events could be detected within the same clone.

Our data here and elsewhere (5, 7) have shown that the in vivo synthesis and in vitro clonal expression of IgG2 Ab to TNP-Ficoll is strongly influenced by the presence of accessory T cells. We have interpreted this as an ancillary effect of T cells on the IgG switching pathway. When T cell effects on the clonal synthesis of IgE and IgA anti-TNP Ab were analyzed, we found that T cells did not significantly increase the clonal frequency of IgE-secreting clones but did significantly increase the frequency of IgA-secreting clones. It should be noted that, unlike IgG2a-positive clones, which were very infrequently found in the absence of T cells, IgA-positive clones did constitute a large proportion of total positive clones in fragments lacking T lympho-cytes. It appears that T cells primarily increase the incidence of clones that secrete IgA only. Whether they do so by enhancing the immature B cell switch to IgA (18, 20) or by helping to trigger mature IgA-positive B cell precursors is not clear.

A number of other investigators (34-37) have shown IgE synthesis to be a very T cell-dependent phenomenon. It is possible that our system fails to show this because (a) TNP-Ficoll is capable of directly inducing a switch to IgE in the splenic focus assay and (b) the population of unprimed donor T cells that we used may be devoid of IgE-specific helper cells or may contain a population of suppressor cells for IgE synthesis.

Although results from the splenic focus assay indicated that a considerable number of TNP-Ficoll-stimulated B cell clones produced IgE and IgA anti-TNP Ab, results from an analysis of serum of immune mice revealed minimal or no IgA and IgE anti-TNP Ab. A relatively high production of IgA Ab in splenic fragment cultures has also been noted in other laboratories (21, 38, 39). Because the variation in results between serum analysis and in vitro clonal analysis is so striking, it is worth discussing some possible explanations. (a) Although the relatively short in vivo half-lives of IgE and IgA (14, 15, 40) may contribute slightly to the low IgE and IgA serum antibody levels measured, differential serum clearance cannot be the major explanation. This is shown by the fact that the in vitro secretion of IgE and IgA Ab from in vivo immunized spleen cells was also found to be lower than the secretion of other isotypes (unpublished observations). (b) The observed lower average production of IgE within positive clones may contribute somewhat to the fact that IgE was undetectable in serum of immune mice but detectable in the splenic focus assay. However, because the average production of IgA within positive clones was higher than that of IgG1 or IgG2b, such an explanation cannot be responsible for the fact that IgA Ab was so poorly represented in immune serum yet well represented in the splenic focus assay. (c) The signals that favor switches to IgE and IgA production may be more adequately delivered in in vitro cultured splenic fragments than in vivo. (d) In vivo, the sites of IgE and IgA Ab secretion vs. IgM and IgG Ab secretion may differ. Indeed, evidence exists that the circulatory patterns of IgE- and IgA-bearing B cells may be distinct from those of IgM- or IgG-bearing B cells (41–44). Thus, it is possible that, in vivo, progeny of TNP-Ficoll responsive splenic precursor cells which switch into IgE or IgA production may emigrate from the spleen, relocalize in intestinal lymphatic tissue, and secrete their Ab in the gut rather than into the serum. The relatively higher IgE/IgG and IgA/IgG ratios observed in the splenic focus assay may be explained by the fact that this system precludes cellular migration.

In concluding this discussion, we should note that the response of B lymphocytes to TNP-Ficoll appears to involve only one of the two principal B cell subsets, Lyb-5$^+$ B cells. Furthermore, this response appears to be independent of carrier-specific helper T cells.[4] Thus, the conclusions concerning the nature of switching events within these clones should be limited to responses of these type. Other switching "pathways," of quite different nature, may be used in responses to classical thymus-dependent or type 1 antigens.

Summary

The IgM, IgG subclass, IgE, and IgA anti-trinitrophenyl (TNP) antibody (Ab) response of B cells to the type 2 antigen TNP-Ficoll was studied in athymic nude mice and in the in vitro splenic focus assay. Results from the splenic focus assay in which purified B lymphocyte preparations had been transferred to irradiated nu/nu recipients indicate that many TNP-Ficoll stimulated B cell clones secrete multiple isotypes and hence appear to be undergoing intraclonal isotype switching. Although the frequency of clones secreting each of the IgG subclasses was found to correlate with 5' to 3' *Igh-γ* gene order, the frequency of IgE and IgA-secreting clones did not

appear to be influenced by the respective position of *Igh-ε* and *Igh-α* on the chromosome. Unlike clones that secreted anti-TNP Ab of the IgG subclasses, IgE and IgA anti-TNP Ab-secreting clones did not have a high propensity for coexpression of isotypes encoded by 5' *Igh-C* genes. These data suggest that three distinct switching pathways may be employed by B cells responding to TNP-Ficoll: a common IgG pathway, an IgE pathway, and an IgA pathway. The presence of T cells resulted in a preferential enhancement of the production of anti-TNP Ab of those IgG subclasses which were least represented in the absence of T cells, i.e., IgG2b and IgG2a. No significant enhancement of IgE anti-TNP clonal frequency was found in the presence of T lymphocytes, but T cells were found to significantly enhance the clonal expression of IgA anti-TNP Ab. Although a relatively large number of B cell clones were found to synthesize IgE and IgA anti-TNP Ab in the splenic focus assay, relatively little or no secretion of these isotypes was detected in immune mice. Possible explanations for this apparent discrepancy are discussed.

References

1. Shimizu, A., N. Takahashi, Y. Yaoita, and T. Honjo. 1982. Organization of the constant-region gene family of the mouse immunoglobulin heavy chain. *Cell.* **28:**499.

2. Liu, C. P., P. W. Tucker, J. F. Mushinski, and F. R. Blattner. 1980. Mapping of heavy chain genes for mouse immunoglobulins M and D. *Science (Wash. D. C.).* **209:**1348.

3. Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa. 1980. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. *Nature (Lond.).* **286:**676.

4. Alt, F. W., N. Rosenberg, R. J. Casanova, E. Thomas, and D. Baltimore. 1982. Immunoglobulin heavy-chain expression and class switching in a murine leukaemia cell line. *Nature (Lond.).* **296:**325.

5. Mongini, P. K. A., W. E. Paul, and E. S. Metcalf. 1982. T cell regulation of immunoglobulin class expression in the antibody response to trinitrophenyl-Ficoll: evidence for T cell enhancement of the immunoglobulin class switch. *J. Exp. Med.* **155:**884.

6. Mosier, D. E., I. M. Zitron, J. J. Mond, A. Ahmed, I. Scher, and W. E. Paul. 1977. Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. *Immunol. Rev.* **37:**89.

7. Mongini, P. K. A., K. E. Stein, and W. E. Paul. 1981. T cell regulation of IgG subclass antibody production in response to T-independent antigens. *J. Exp. Med.* **153:**1.

8. Mond, J. J., P. K. A. Mongini, D. Sieckmann, and W. E. Paul. 1980. Role of T lymphocytes in the response to TNP-AECM-Ficoll. *J. Immunol.* **125:**1066.

9. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* **15:**47.

10. Kung, J., S. Sharrow, C. Thomas, and W. Paul. 1982. Analysis of B lymphocyte differentiation antigens by flow microfluorometry. *Immunol. Rev.* In press.

11. Yutoku, M., A. L. Grossberg, R. Stout, L. A. Herzenberg, and D. Pressman. 1976. Further studies on Th-B, a cell surface antigenic determinant present on mouse B cells, plasma cells and immature thymocytes. *Cell. Immunol.* **23:**140.

12. Mongini, P. K. A., and E. Heber-Katz. 1982. Use of a solid phase ^{3}H radioimmunoassay for the measurement of immunoglobulin produced in short term cultures of antibody-secreting-cells. *J. Immuno. Methods.* **49:**39.

13. Wilder, R. L., C. C. Yuen, B. Subbarao, V. L. Woods, C. B. Alexander, and R. G. Mage. 1979. Tritium (^{3}H) radiolabeling of protein A and antibody to high specific activity: application to cell surface antigen radioimmunoassays. *J. Immunol. Methods.* **28**:255.

14. Orlans, E., J. Peppard, J. Reynolds, and J. Hall. 1978. Rapid active transport of immunoglobulin A from blood to bile. *J. Exp. Med.* **147**:588.

15. Waldmann, T. A. 1969. Disorders of immunoglobulin metabolism. *N. Engl. J. Med.* **281**:1170.

16. Gearhart, P. J., N. H. Sigal, and N. R. Klinman. 1975. Production of antibodies of identical idiotype but diverse immunoglobulin classes by cells derived from a single stimulated B cell. *Proc. Natl. Acad. Sci. U. S. A.* **72**:1707.

17. Gearhart, P. J., J. L. Hurwitz, and J. J. Cebra. 1980. Successive switching of antibody isotypes expressed within the lines of a B cell clone. *Proc. Natl. Acad. Sci. U. S. A.* **77**:5424.

18. Teale, J. M., D. Lafrenz, N. R. Klinman, and S. Strober. 1981. Immunoglobulin class commitment exhibited by B lymphocytes separated according to surface isotype. *J. Immunol.* **126**:1952.

19. Teale, J. M., F.-T. Liu, and D. H. Katz. 1981. A clonal analysis of the IgE response and its implications with regard to isotype commitment. *J. Exp. Med.* **153**:783.

20. Teale, J. M., and T. E. Mandel. 1980. Ontogenetic development of B lymphocyte function and tolerance susceptibility in vivo and in an in vitro fetal organ culture system. *J. Exp. Med.* **151**:429.

21. Hurwitz, J. L., V. B. Tagart, P. A. Schweitzer, and J. J. Cebra. 1982. Patterns of isotype expression by B cell clones responding to thymus-dependent and thymus-independent antigens *in vitro. Eur. J. Immunol.* **12**:342.

22. Davis, M. M., S. M. Kim, and L. E. Hood. 1980. DNA sequences mediating class switching in α-immunoglobulins. *Science (Wash. D. C.).* **209**:1360.

23. Kuritani, T., and M. D. Cooper. 1982. Human B cell differentiation. I. Analysis of immunoglobulin heavy chain switching using monoclonal anti-immunoglobulin M, G, and A antibodies and pokeweed mitogen-induced plasma cell differentiation. *J. Exp. Med.* **155**:839.

24. Ishizaka, K., M. Suemura, J. Yodoi, and M. Hirashima. 1981. Regulation of IgE response by IgE binding factors. *Fed. Proc.* **40**:2162.

25. Jarrett, E., and H. Bazin. 1974. Elevation of total serum IgE in rats following helminth parasite infection. *Nature (Lond.).* **251**:613.

26. Kishimoto, T., and K. Ishizaka. 1973. Regulation of antibody response in vitro. VI. Carrier-specific helper cells for IgG and IgE antibody response. *J. Immunol.* **111**:720.

27. Kimoto, M., T. Kishimoto, S. Noguchi, T. Watanabe, and Y. Yamamura. 1977. Regulation of antibody response in different immunoglobulin classes. II. Induction of *in vitro* IgE antibody response in murine spleen cells and demonstration of a possible involvement of distinct T helper cells in IgE and IgG antibody responses. *J. Immunol.* **118**:840.

28. Katz, D. H. 1980. Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. *Immunology.* **41**:1.

29. Tada, T. 1975. Regulation of reaginic antibody formation in animals. *Prog. Allergy.* **19**:122.

30. Fox, D. A., N. Chiorazzi, and D. H. Katz. 1976. Hapten specific IgE antibody responses in mice. V. Differential resistance of IgE and IgG B lymphocytes to X-irradiation. *J. Immunol.* **117**:1622.

31. Chiorazzi, N., D. A. Fox, and D. H. Katz. 1976. Hapten-specific IgE antibody responses in mice. VI. Selective enhancement of IgE antibody production by doses of X-irradiation and by cyclophosphamide. *J. Immunol.* **117**:1629.

32. Kishimoto, T., S. Shigemoto, T. Watanabe, and Y. Yamamura. 1979. Demonstration of phosphorylcholine-specific IgE B cells in CBA/N mice. *J. Immunol.* **123**:1039.

33. Durkin, H. G., H. Bazin, and B. H. Waksman. 1981. Origin and fate of IgE-bearing

lymphocytes. I. Peyer's patches as differentiation site of cells simultaneously bearing IgA and IgE. *J. Exp. Med.* **154:**640.

34. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J. Immunol.* **106:**1019.

35. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1973. Hapten-specific IgE antibody responses in mice. II. Cooperative interactions between adoptively transferred T and B lymphocytes in the development of IgE responses. *J. Exp. Med.* **138:**538.

36. Ishizaka, K., and H. Okudaira. 1973. Reaginic formation in the mouse. II. Enhancement and suppression of anti-hapten antibody formation by priming with carrier. *J. Immunol.* **110:**1067.

37. H. Bazin, B. Platteau, R. Pauwels, and A. Capron. 1980. Immunoglobulin production in nude rats with special attention to the IgE isotype. *Ann. Immunol. (Inst. Pasteur).* **131C:**31.

38. Nakamura, I., A. Ray, and O. Makela. 1973. Oligomeric IgA: the major component of the in vitro primary response of mouse spleen fragments. *J. Exp. Med.* **138:**973.

39. Pasanen, V. J., K. Karjalainen, M. Kaartinen, and O. Makela. 1981. Preferential production of IgA antibodies by spleen fragments immunized and maintained in vitro. *Scand. J. Immunol.* **13:**111.

40. Jackson, G. D. F., I. Lemaitre-Coelho, J. -P. Vaerman, H. Bazin, and A. Beckers. 1978. Rapid disappearance from serum of intravenously injected rat myeloma IgA and its secretion into bile. *Eur. J. Immunol.* **8:**123.

41. Ishizaka, K., and T. Ishizaka. 1978. Mechanism of reaginic hypersensitivity and IgE-antibody response. *Immunol. Rev.* **41:**109.

42. Parrott, D. H. V. 1976. The gut as a lymphoid organ. *Clin. Gastroenterol.* **5:**211.

43. Weisz-Carrington, P., M. E. Roux, M. McWilliams, J. M. Phillips-Quagliata, and M. E. Lamm. 1979. Organ and isotype distribution of plasma cells producing specific antibody after oral immunization: evidence for a generalized secretory immune system. *J. Immunol.* **123:**1705.

44. McDermott, M. R., and J. Bienenstock. 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. *J. Immunol.* **122:**1892.