MEMORY B CELLS AT SUCCESSIVE STAGES OF DIFFERENTIATION

Affinity Maturation and the Role of IgD Receptors* ‡

By LEONORE A. HERZENBERG, SAMUEL J. BLACK,§ TAKESHI TOKUHISA, AND LEONARD A. HERZENBERG

From the Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

The antigen-induced development of memory B cell populations involves both an increase in the number of B cells capable of responding to an antigen and, especially with time and repeated antigen stimulation, a selective expansion of B cells committed to producing antibodies with higher combining affinities for the antigen (1). These two events, memory generation and affinity maturation, have generally been considered to be different manifestations of the same ongoing selection-expansion process; however, studies presented here demonstrate that they occur at different stages during memory development and can be regulated independently. In addition, these studies suggest that the presence or absence of surface IgD receptors may play a crucial role in determining the potential for affinity maturation in memory populations.

We have shown that both IgD^+ and IgD^- [1] B cells from primed donors give rise to adoptive IgG memory responses and that IgD^+ memory cells are precursors of IgD^- memory cells (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.) (2, 3). In this paper, we further define the relationship between these memory populations by showing that IgD^+ memory cells taken from donors that were primed under a variety of conditions always give rise to predominantly low-affinity adoptive responses, whereas their progeny, IgD^- memory cells, in the same donors, uniformly give rise to higher-affinity responses that are characteristic of secondary responses to strong antigenic stimuli. These data, which support and extend conclusions from our earlier studies (2, 3), indicate that affinity maturation initiates with the emergence of the IgD^- memory population and proceeds mainly, if not exclusively, within this population thereafter. Thus, the transition from early (IgD^+) to mature (IgD^-) memory is required for the development of high-affinity response.

Data presented here also bear on the roles played by helper T cells during memory development. Earlier studies have shown that affinity maturation is regulated by the

* Supported in part by National Institutes of Health grants HD-01287 and CA-04681.

‡ Preliminary reports of this study were presented at the "B Lymphocytes in the Immune Response" meeting held in Scottsdale, Arizona, 14–18 October 1978.

§ Senior Fellow of the California Division of the American Cancer Society.

[1] The term IgD^- denotes cells with no detectable surface IgD by fluorescence-activated cell sorter analysis. The lower limit of detection for IgD as measured here is $\sim 10^4$ surface IgD molecules, from the F:P ratio of fluorescein-conjugated anti-IgD reagent used and calibration values for fluorescein detection on our fluorescence-activated cell sorter instrument.

availability of helper T cells. Gershon and Paul (4) demonstrated a marked reduction in the capacity for development of high-affinity responses in T-depleted animals reconstituted with limiting T cell doses. We showed an apparently similar impairment in affinity maturation in the 1b allotype[2] memory population in mice depleted (by allotype suppressor T cells) of helper T cells required specifically to help 1b allotype B cells (5). Studies presented here extend these latter observations by showing that the failure in affinity maturation in allotype-suppressed mice a result of the selective inability of the suppressed environment to support a normal transition from an IgD^+ population to a predominantly IgD^- population. These studies, coupled with the demonstration that carrier-specific helper T cells are required for the expansion of IgD^- memory populations (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.) provide a partial understanding of the mechanisms through which T cells regulate memory B cell development and thus regulate the quality as well as the quantity of antibody produced.

Materials and Methods

Mice. SJL/J, BALB/c, and (BALB/c × SJL)F_1 hybrid mice were used in this study. Allotype-suppressed mice were BALB/c × SJL born to BALB/c mothers actively producing anti-Igh-1b (6). BALB/c carries the Igh^a immunoglobulin heavy-chain-region haplotype. SJL carries the Igh^b haplotype.

Allotype Notation. Our previous allotype notation is replaced here by a new notation system recently agreed upon by investigators in the field. Igh-1a and Igh-1b (previously called Ig-1a and Ig-1b) are allotypes of IgG_{2a} isotype immunoglobulins; Igh-4a and Igh-4b are IgG_1 allotypes; and Igh-5a and Igh-5b are IgD allotypes. For simplicity, these allotypes are referred to as 1a, 1b, etc., in the text.[3]

Anti-IgD Sera. Studies with BALB/c × SJL hybrids were performed with monoclonal IgM anti-5b antibodies (H6/31) received as a gift from Dr. T. Pearson and Dr. C. Milstein, Medical Research Council, Cambridge, England.

Homogeneous Anti-2,4-Dinitrophenyl (DNP) Antibodies. MOPC-460 was received from Dr. P. A. Casenave, Pasteur Institute, Paris, France, and MOPC-315 was received from Dr. I. Weissman, Stanford University, Stanford, Calif. Monoclonal anti-DNP antibodies 3-12-D and 6-16-A were provided by M. Scott and Dr. J. Fleischman, Washington University Medical School, St. Louis, Mo. Monoclonal antibody 29-B5 was generated in our laboratory (Stanford University School of Medicine, Stanford, Calif.).

Priming. B cell donors were (hapten) primed with 100 μg DNP-chicken gamma globulin (CGG), with DNP-keyhole limpet hemocyanin (KLH) or with 800 μg DNP-bovine serum albumin (BSA). T cell donors were carrier-primed with 100 μg CGG or KLH. Except where stated, all antigens were injected intraperitoneally as alum precipitates together with 2×10^9 killed *Bordetella pertussis* organisms (Department of Public Health, Boston, Mass.).

Cytotoxic Depletion for Cell Transfers. A 1:500 dilution of ascites fluid that contained the H6/

---

[2] See Materials and Methods: Allotype Notation.

[3] *Abbreviations and nomenclature used in this paper:* afc, antibody-forming cell(s); BSA, bovine serum albumin; CGG, chicken gamma globulin; CTh; carrier-specific T helper cell(s); DNP, 2,4-dinitrophenyl; FACS, fluorescence-activated cell sorter; F*, fluorescein conjugated; IgTh, Ig-specific T helper cell(s); K_a binding affinity constant; KLH, keyhole limpet hemocyanin; RIA, solid-phase radioimmune binding assay(s); Th, T helper cell(s); Ts, suppressor T cell(s); 1a (Igh-1a), *a* heavy-chain allotype of IgG_{2a} isotype immunoglobulin; 1b (Igh-1b), *b* heavy-chain allotype of IgG_{2a} isotype immunoglobulin; 4a (Igh-4a), *a* heavy-chain allotype of IgG_1 isotype immunoglobulin; 4b (Igh-4b), *b* heavy-chain allotype of IgG_1 isotype immunoglobulin; 5a (Igh-5a), *a* heavy-chain allotype of IgD isotype immunoglobulin; 5b (Igh-5b), *b* heavy-chain allotype of IgD isotype immunoglobulin.

31 IgM anti-Igh-5b (IgD) was used to deplete Igh-5b-bearing cells. Rabbit anti-mouse brain that was absorbed with cells from a B cell lymphoma until it had no more reactivity against splenic B cells, was used to deplete T cells. Spleen cells (2×10^7/ml) were incubated for 30 min at 4°C with the antibodies at the indicated dilution in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) that contained 0.1% NaN_3. Cells were then washed, resuspended in RPMI (+ NaN_3) that contained 10% guinea pig serum (absorbed first with spleen cells), and incubated for 40 min at 37°C.

Immunofluorescent Staining and Fluorescence-activated Cell Sorting for Cells with Surface IgD. IgD-allotype ($5b^+$) cells were stained directly by reacting BALB/c × SJL spleen cells with fluorescein-conjugated (F*) H6/31 monoclonal anti-5b (F* anti-Igh-5b). This reagent stained 18% of BALB/c × SJL spleen cells and <2% of BALB/c spleen cells.

With the fluorescence-activated cell sorter (FACS), 5b-bearing cells were sorted on the basis of the amount of F* anti-Igh-5b bound. Low-angle light scatter (size) gates were set so that only small, live lymphocytes were analyzed or separated (7). About 5% of spleen cells that spanned the inflection point of the linear-staining profile were discarded to avoid contamination with IgD^- cells. Nearly all Igh-$5b^-$ (IgD^-) cells fell within the first 2 of 240 possible channels. IgD^+ cells were taken above the 30th channel.

Adoptive Secondary Responses. T-depleted B cells (unseparated, Igh-5b depleted, FACS-sorted Igh-$5b^+$ or FACS-sorted Igh-$5b^-$), recovered from 10^7 spleen cells from DNP-primed normal or DNP-primed Igh-1b allotype-suppressed BALB/c × SJL mice, were mixed with 5×10^6 nylon-passed KLH-primed T cells and 10 μg DNP-KLH just before intravenous injection into x-irradiated (650 rads, 18 h previously) BALB/c recipients. Anti-DNP activity in recipient sera that was collected 7 d after transfer was taken as the index of the amount of B cell memory present in the donor B cell population.

Measurement of the Magnitude and Affinity of Serum Anti-DNP Responses. The solid-phase radioimmune binding assay (RIA) used here to measure allotype and class representation in antisera (including the preparation of reagents for this assay) has been described in detail elsewhere (8). Briefly, the assay is performed in 96-well microtiter plates at room temperature in an RIA diluent (1% BSA, 0.005 M EDTA, and 0.1% NaN_3 in phosphate-buffered saline [pH 7.6]) that is also used for washing (three times in between each assay step). First, 50 μl of DNP-BSA (either $DNP_{12}BSA$ or $DNP_{42}BSA$) at 0.05 mg/ml is incubated for 1 h in the wells. Next, test or standard antisera at various dilutions are added to the coated wells (20 μl/well) and incubated for 1 h. Finally, ^{125}I-labeled anti-allotype (20 μl of 1a, 1b, 4a, or 4b, that contained ~2×10^5 cpm/well) is added and incubated for 1 h. Plates are then washed, dried, and cut so that individual wells can be counted in tubes in a gamma scintillation counter.

^{125}I-counts bound to wells are converted to units of antibody response relative to the amounts of anti-DNP antibody of each allotype present in a standard adoptive secondary antiserum by comparison with $DNP_{12}BSA$-binding curves generated from the standard serum in each assay. This method corrects for minor inefficiencies in binding of second step antiallotype reagents and for different ^{125}I-specific activities in these reagents. It allows comparability between results of assays done at different times.

The ratio of units bound to $DNP_{12}BSA$ per units bound:$DNP_{42}BSA$ provides a reliable measure of the average affinity of anti-DNP antibody in test samples. Data that validate this novel affinity assay are presented in Results.

Results

IgD^+ Memory Cells are Precursors of IgD^- Memory Cells. FACS double transfer experiments that are presented in detail elsewhere (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.) show directly that IgD^+ and IgD^- memory cells give rise to IgG responses and that IgD^+ memory cells give rise to IgD^- memory cells. These experiments, summarized in Fig. 1, also show that IgD^- memory cells "self renew" in that they give rise to more IgD^- memory cells capable of transferring memory responses to new adoptive recipients.

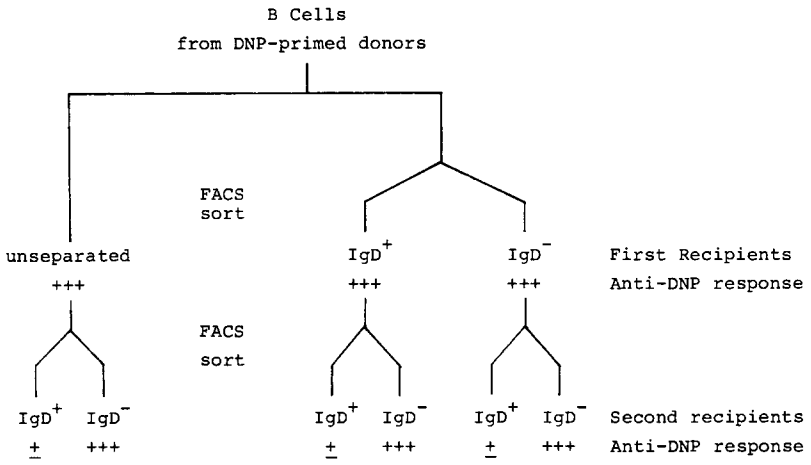

FIG. 1. IgD^+ memory cells give rise to IgD^- memory cells, and IgD^- memory cells self renew. Recipients of B cell populations were irradiated and supplemented with carrier-primed T cells and antigen. Control recipients that were given carrier-primed T and antigen showed ± responses. +++, 60–75 U of IgG anti-DNP antibody; ±, 5–20 U.

The IgD^+ and IgD^- memory populations were FACS-sorted from primed donors and transferred to first recipients that were supplemented with antigen and carrier-primed T cells. Both populations gave rise to IgG responses. 5 wk later, splenic B cell populations that were taken from each group of the first recipients were FACS-sorted into IgD^- and IgD^+ populations, supplemented with carrier-primed T cells and antigen, and transferred to second recipients to determine the fate of the originally transferred IgD^+ and IgD^- memory cells. Responses in the second recipients showed that the IgD^+ and IgD^- memory populations from the original donor had each given rise to substantiate memory populations in the first recipients and that these progeny memory populations consisted almost entirely of IgD^- memory cells regardless of whether they were derived orginally from the IgD^+ or the IgD^- parent population.

Newly Developed RIA Measure the Magnitude and Affinity of Anti-DNP Adoptive Responses. Critical evaluation of the affinity maturation studies presented here requires interpretation of data obtained with a newly developed set of RIA that measure anti-DNP response magnitude and affinity for antibodies of various allotypes present in individual or pooled serum samples from adoptive recipients. To demonstrate the properties of these assays, the binding activities of a series of anti-DNP antisera, monoclonal antibodies, and myeloma proteins that span a broad affinity range are shown in Table I.

The hapten-substitution ratio of the conjugate used as RIA plate-coat antigen is particularly important for the detection of low-affinity antibodies such as those produced in adoptive primary or early (IgD^+) memory responses. As data in Table I show, these antibodies, like the lower-affinity myeloma proteins used for comparison (i.e., MOPC-460 and MOPC-315) are bound and retained better by $DNP_{42}BSA$ than by $DNP_{12}BSA$. Higher-affinity antibodies, in contrast, are bound and retained equally well by the two conjugates or even, with very high affinity antibody, better by $DNP_{12}BSA$ than by $DNP_{42}BSA$.

Binding data shown in Table I are expressed in units that indicate the antigen

TABLE I

Magnitude and Affinity of Anti-DNP Responses Measured By RIA

Anti-DNP antibodies			RIA binding activity*		Affinity for ε-DNP-lysine‡
Source of antibody	Splenic B cells transferred§	Allotype‡‖ or isotype	DNP_{12}	DNP_{42}	K_a M^{-1} ($\times 10^6$)
Myeloma MOPC-460	—	IgA	6	195	0.07
IgD^+ adoptive 2°	Short-term 1° FACS IgD^+	Igh-1b	20	75	(0.35)¶
		Igh-4b	23	82	(0.35)
Monoclonal 3-12-D	—	Igh-4e	42	110	1.0
T-helped adoptive 1°	Unprimed	Igh-1b	12	25	(1.4)
Myeloma MOPC-315	—	IgA	100	195	2
Adoptive 2° (standard)	Long-term 1°	Igh-1a	100	130	(9)
		Igh-1b	100	120	(20)
		Igh-4a	100	160	(4)
		Igh-4b	100	130	(9)
IgD^- adoptive 2°	Short-term 1° FACS IgD^-	Igh-1b	65	64	(50)
		Igh-4b	70	70	(50)
Hyperimmune	1° and boosted *in situ* response	Igh-1b	180	160	(90)
Monoclonal 6-16-A		Igh-4e	146	128	110
Monoclonal 29-B5	—	Igh-4b	370	280	300

* RIA binding activities to $DNP_{12}BSA$ and $DNP_{42}BSA$ are expressed in relative units based on amount of binding of the appropriate allotype obtained on $DNP_{12}BSA$ with the (standard) adoptive secondary antiserum shown in the table. This serum contains ~0.1 mg/ml Igh-1b anti-DNP antibody. Levels of Igh-4a and Igh-4b anti-DNP antibodies are probably 10-fold higher. MOPC-315 binding to $DNP_{12}BSA$ was used for the IgA standard because the adoptive 2° standard serum contained virtually no detectable IgA anti-DNP.

‡ K_a values for myeloma proteins and monoclonal antibodies were measured by determining the concentration of ε-DNP-lysine required to quench 50% of the maximum quenchable fluorescence of purified monoclonal antibody at a known protein concentration. Values for MOPC-315 and MOPC-460 are consistent with previously published values (9, 10). Measurements were kindly performed by Dr. J. W. Parce in Dr. Harden McConnell's laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

§ All B cell populations were T depleted before transfer and supplemented with carrier-primed nylon T plus antigen; sera tested were taken 7 d after transfer. FACS-separated responses were from SJL mice; other responses were from (SJL × BALB/c)F_1 mice.

‖ For isotype correspondence, see Materials and Methods.

¶ Average affinities for response sera (shown in parentheses) were estimated from the curve shown in Fig. 2.

binding capacity of the test serum relative to the binding capacity of a standard adoptive secondary antiserum on $DNP_{12}BSA$. This serum contains ~0.1 mg/ml of 1b anti-DNP, an apparently similar amount of 1a anti-DNP, and probably ~10 times as much antibody of each of the IgG_1 allotypes. Units of response can be taken as proportional to these values except when test affinities vary more than an order of magnitude from the average affinity of the standard serum.

Differential RIA Binding to $DNP_{12}BSA$ and DNP_{42} Measures Average Response Affinity. Determination of the ratio of units of antibody bound to $DNP_{12}BSA$ and $DNP_{42}BSA$ allows determination of the average affinity of the anti-DNP antibodies in a given antiserum. This ratio, measured for a series of homogenous anti-DNP

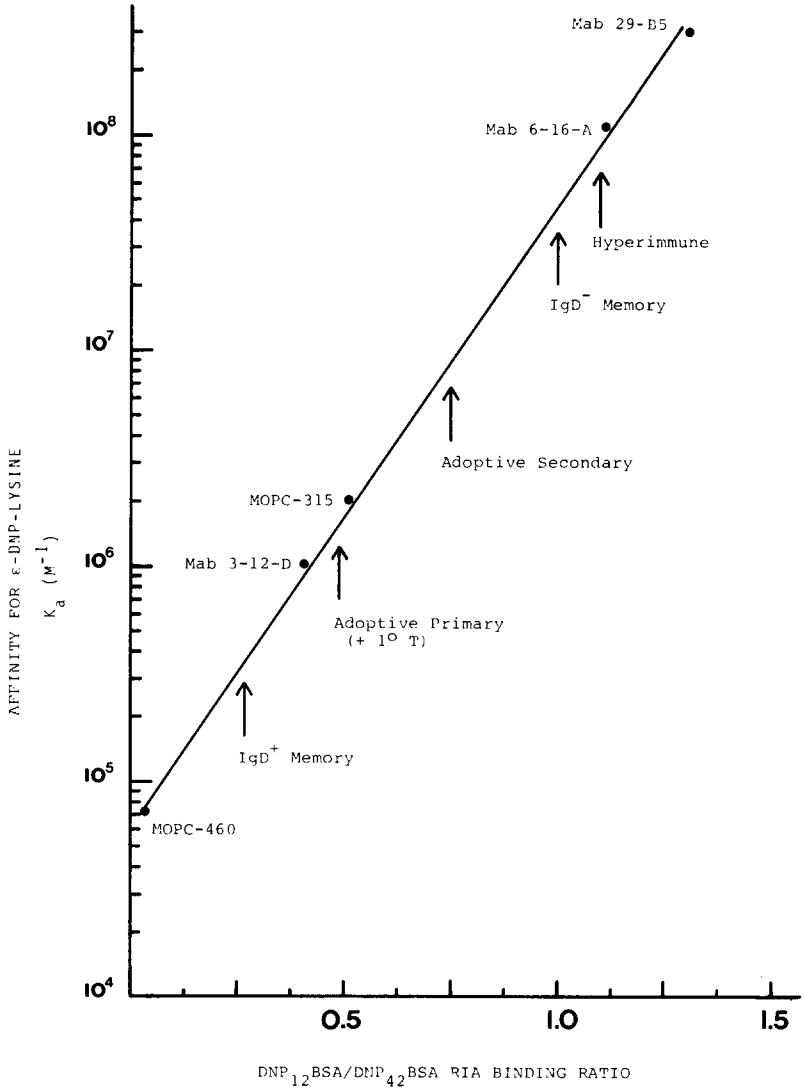

FIG. 2. Ratio of RIA binding to differently substituted DNP-BSA conjugates indicates anti-DNP antibody K_a. K_a values (points) for myeloma proteins and monoclonal antibodies were determined by fluorescence quenching (Table I, footnote). Arrows indicate $DNP_{12}BSA$:$DNP_{42}BSA$ ratios for various immune responses. Complete data are shown in Table I.

antibodies (myeloma proteins and monoclonal antibodies), is proportional to the log of the binding affinity constant (K_a) of the antibody (Fig. 2). The proportionality maintains over more than three orders of affinity magnitude and spans essentially the entire range of affinities normally obtained in *in situ* and adoptive anti-DNP responses (Fig. 2, arrows). The average affinities of various responses can thus be read from the "standard curve" generated by the homogeneous antibodies. These affinities are shown in Table I.

Data reported in subsequent tables in this publication are based on RIA binding measurements for 1a, 1b, 4a, and 4b anti-DNP on $DNP_{12}BSA$ and $DNP_{42}BSA$. Units

TABLE II

B Cell Memory Shift from IgD^+ to IgD^- with Time After Priming

BALB/c × SJL spleen DNP 1° (alum + pertussis) (weeks after priming)	Igh-1b anti-DNP adoptive secondary response (day 7)			
	All B cells*		IgD^- B cells	
	Anti-DNP‡ response	Mean K_a M^{-1}§ ($\times 10^{-6}$)	Anti-DNP‡ response	Mean K_a M^{-1}§ ($\times 10^{-6}$)
Unprimed	2		<1	
1	15	<0.1	<1	
2	75	3	40	12
4	126	100	102	100
6	121	50	115	50
10	40	50	ND	ND

* All B cells: T-depleted B cells (from 10^7 spleen) supplemented with 5×10^6 carrier-primed (nylon-passed) T + 10 μg DNP-KLH i.v., B cells taken 4 wk after priming. IgD^- B cells: same with IgD^+ cells depleted (Materials and Methods).

‡ RIA binding on $DNP_{42}BSA$ is expressed relative to adoptive secondary standard binding.

§ Estimated from curve in Fig. 1.

of $DNP_{42}BSA$ binding are shown as, an index of response magnitude. The K_a values corresponding to the DNP_{12}:DNP_{42} ratio observed for each allotype, read from the standard curve in Fig. 1, are shown in the tables as an estimation of the average affinity of the response (mean K_a). Each set of these measurements (four allotypes per sample) required somewhere between 0.1 and 2.0 μl of test serum. Because roughly 30 sets of measurements can be easily made in a single assay with the appropriate equipment, this relatively simple method for distinguishing low-affinity from higher-affinity responses enables rapid assay of antibodies in large numbers of serum samples and makes studies such as those presented here feasible.

A Variety of Parameters Control the Proportions of IgD^+ and IgD^- Cells in Memory Populations. Memory that is attributable to IgD^+-primed B cells was measured for this series of experiments by comparing adoptive responses from total B cell populations with responses from populations depleted for IgD^+ cells by cytotoxic treatment with monoclonal anti-IgD allotype reagents (anti-5b). All populations were T depleted and supplemented with carrier-primed (nylon-passed) T cells and 10 μg DNP-KLH when transferred. Data are shown for the 1b (IgG_{2a}) allotype anti-DNP responses in adoptive recipients 7 d after transfer. Data for 4b allotype responses were consistent with the 1b results shown. The 1a and 4a responses were not affected by depletion of 5b-bearing precursors because, as a result of haplotype exclusion, these cells give rise only to *b* allotype IgG responses.

B cells from unprimed donors show essentially no detectable IgG response under the (adoptive secondary) conditions used here (Table II). Thus, the B cell populations that do respond have arisen in response to primary antigenic stimulation of the donor and are appropriately classified as memory cells.

Comparison of responses from IgD^+-depleted and -intact memory populations shows that the transition from IgD^+ to IgD^- memory occurs progressively with time after priming (Table II). Similar comparisons show that details of priming protocols (adjuvant and carrier protein) also significantly influence the rate of this transition (Table III), i.e., *B. pertussis* accelerates the development of IgD^- memory cells; alum

TABLE III

Carrier and Adjuvant Both Influence the Rate of Affinity Maturation

DNP priming of BALB/c × SJL B cell donor		Igh-1b adoptive secondary response (day 7)			
		All B cells*		IgD⁻ B cells	
Antigen	Adjuvant	Anti-DNP‡ response	Mean K_a (M^{-1})§ ($\times 10^{-6}$)	Anti-DNP‡ response	Mean K_a (M^{-1})§ ($\times 10^{-6}$)
DNP-KLH	Alum + pertussis	120	15	112	25
	Alum	83	1.5	50	50
	Freund's	78	2	30	110
DNP-BSA	Freund's	34	0.2	6	25

* All B cells: T-depleted B cells supplemented with carrier-primed (nylon-passed) T + 10 μg DNP-KLH i.v., B cells taken 4 wk after priming. IgD⁻ B cells: same with IgD⁺ cells depleted (Materials and Methods).

‡ RIA binding on $DNP_{42}BSA$ is expressed relative to adoptive secondary standard binding.

§ Estimated from curve in Fig. 1.

and Freund's complete adjuvant are less effective in this regard; and KLH, as shown previously (4), is a much more effective carrier than BSA for generating higher-avidity IgD⁻ memory. Finally, genetic factors are important in determining the rate of IgD⁺ to IgD⁻ transition. For example, the memory pool in SJL mice contains a sizable IgD⁺ component, whereas the memory pool in similarly primed BALB/c × SJL mice is almost all IgD⁻ (compare results from FACS-isolated SJL memory populations shown in Table I with BALB/c × SJL data from 6-wk-primed mice shown in Table II).

Affinity Maturation Occurs Mainly Within IgD⁻ Memory Populations. Comparison of the average affinities for responses from IgD-depleted and intact memory populations demonstrate that emerging IgD⁻ memory populations give rise to substantially higher-affinity responses than IgD⁺ memory populations present in the same donor (Tables II and III). Data presented are representative of data from a larger series of cytotoxic depletion experiments that show that whenever depletion of IgD⁺ cells reduces a memory response, it selectively removes the low-affinity component of the response. Regardless of the age, sex, strain, or priming history of the donor, and regardless of whether the depleted IgD⁺ cells constitute a very large or very small proportion of the total memory population, the residual (IgD⁻) memory cells always generate responses in the higher-affinity range that is characteristic of mature memory populations.

FACS-isolation studies complement this series of experiments by directly demonstrating that isolated IgD⁺ memory populations from a variety of sources give rise only to low-affinity responses. Data in Table I, for example, show the low-affinity response obtained with IgD⁺ memory cells from an SJL donor primed 6 wk previously with 100 μg of DNP-KLH plus *B. pertissus*. Data in Table IV show similar low-affinity responses with IgD⁺ cells taken from primed and boosted allotype-suppressed mice (where the IgD⁺ to IgD⁻ transition for the 1b memory population is impaired by the suppression).

The FACS-isolated IgD⁻ memory populations from these donors again gave rise to higher-affinity responses (Tables I and IV). Thus, data from FACS-isolation and IgD⁺-depletion studies are consistent in showing that IgD⁺ memory populations, no

TABLE IV

IgD^+ to IgD^- Transition Is Retarded for Igh-1b Memory in Igh-1b Allotype-suppressed Mice

DNP 1° BALB/c × SJL spleen		IgG Anti-DNP adoptive 2° response (day 7)			
		Igh-1b		Igh-4b	
Status	B cells* transferred	Anti-DNP‡ response	Mean K_a (M^{-1})§ ($\times 10^{-6}$)	Anti-DNP‡ response	Mean K_a (M^{-1})§ ($\times 10^{-6}$)
Igh-1b suppressed‖ (boosted three times)	FACS IgD^+	25	2	8	20
	FACS IgD^-	18	50	40	50
Igh-1b suppressed	FACS IgD^+	140	0.4		
	FACS IgD^-	60	50		
Nonsuppressed	FACS IgD^+	1			
	FACS IgD^-	108	50		

* T depleted and supplemented with carrier-primed T.
‡ RIA binding on $DNP_{42}BSA$ is expressed relative to adoptive secondary standard binding.
§ Estimated from curve in Fig. 1.
‖ 1 yr old when tested.

matter how long they persist, show relatively little increase in the affinity of responses that they generate, whereas IgD^- memory populations, no matter how early they appear, still show sufficient affinity maturation to raise the average affinity of the responses they generate substantially above the responses of the parent IgD^+ memory population.

Development of High-Affinity IgD^- Memory Does Not Appear to Selectively Deplete High-Affinity Precursors from Residual IgD^+ Memory Populations. The difference between IgD^+ and IgD^- response affinities is clearest 7 d after transfer when relatively little affinity maturation has taken place in adoptive recipients. 1 wk later, (i.e., at 14 d after transfer), both the IgD^+ and IgD^- response affinities have increased such that, although a disparity still remains, the IgD^+ response affinity roughly equals the response affinity of IgD^- cells at 7 d after transfer (data not shown). Preliminary observations suggest that even when the IgD^+ to IgD^- memory transition is nearly complete and the IgD^+ population represents only a small proportion of the total memory pool, it is still capable of rather rapidly generating a high-affinity response if allowed to mature in an adoptive recipient. Furthermore, IgD^+ populations appear to give rise to extremely high-affinity responses when restimulated in adoptive recipients.

These data suggest that the IgD^+ memory population, so long as it persists, does not become selectively depleted for high-affinity memory cells. Instead, the early memory population appears to shrink evenly without modification of its original distribution of precursor affinities. If anything, long-persisting IgD^+ populations appear to increase somewhat in average affinity. Thus, it is likely that the differentiation from IgD^+ to IgD^- is relatively unselective with respect to affinity and that affinity maturation occurs essentially exclusively by selective expansion within the differentiated IgD^- population.

Affinity Maturation is Regulated by Helper T Cells. We have shown elsewhere (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self

renewal. Manuscript in preparation.) that expansion of IgD^- memory cells requires help from carrier-specific T helper cells (Th)(CTh). Because affinity maturation appears to involve the selective expansion of these memory cells, CTh must be involved in regulating this process. Data from studies presented here also implicate a second T helper cell in the regulation of B cell memory development. These studies show that the transition from IgD^+ to IgD^- memory is hampered in allotype-suppressed mice known to be depleted for a Th population (Ig-specific Th [Ig Th]) that is required, in addition to CTh, for the expression of memory B cells (11).

IgTh have been demonstrated in allotype (11) and idiotype (12, 13) suppression systems and appear to be specific for Ig determinants on the cells they help. In the 1b allotype-suppressed mice used here, a Ts population induced by perinatal exposure to antibody to the (paternal) 1b allotype specifically depletes Ig Th (1b Th) required for the differentiation of 1b memory cells to afc that secrete 1b antibody (11).

Previous studies have shown that 1b-suppressed mice primed and boosted with DNP-KLH develop apparently normal numbers of 1b memory cells but that the affinity of the 1b memory response is substantially lower than the affinity of responses from other IgG memory populations in the same donor (5). The 1b memory population is demonstrable after a single (priming) antigen dose, but remains essentially cryptic *in situ* (because the mice are suppressed) and thus can be demonstrated only by adoptive transfer of T-depleted spleen cell populations supplemented with carrier-primed T cells from syngeneic nonsuppressed mice.

Data presented here confirm these observations and show further that the low-affinity response from the suppressed 1b memory population is a result of the failure of this memory population to mature normally from IgD^+ to IgD^- (Table IV). FACS-separation studies show that the 1b memory population in long-term primed and boosted suppressed mice has a large IgD^+ low-affinity component no longer present in 1b memory populations from similarly primed nonsuppressed mice. In fact, the ratio of IgD^+:IgD^- memory in the 1b memory pool in suppressed mice is even higher than the ratio of IgD^+:IgD^- memory in syngeneic nonsuppressed mice 2 wk after priming (compare Tables II and IV). Other IgG memory populations in suppressed mice, however, show the normal shift to IgD^- with time. The 4b memory population, for example, is essentially IgD^- and shows the usual higher-affinity response obtained with long-term primed memory cells whether taken from suppressed or normal donors (Table IV).

The 1a and 4a memory populations in suppressed and normal mice also show higher-affinity adoptive responses characteristic of IgD^- memory populations. (Data for these responses were omitted from Table IV to avoid confusion because the reagent used to stain cells for separation is specific for 5b, the IgD allotype on haplotype-excluded precursors of Ig^b allotype antibody-forming cell [afc], and therefore does not react with 5a, the IgD allotype on Ig^a precursors.) Thus, all IgG memory populations examined show an affinity maturation pattern commensurate with their priming history except for the 1b population in allotype-suppressed mice. This population alone remains similar in its average affinity to memory populations in recently primed mice and, like these memory populations, retains a large IgD^+ memory component.

The IgD^- 1b memory cells in suppressed mice represent a variable, but consistently detectable, proportion of the memory pool. These cells apparently persist indefinitely and undergo affinity maturation, because their responses in adoptive recipients,

regardless of the proportion of the memory pool they represent, are always in the higher-affinity range characteristic of mature memory populations (Table IV). Therefore, the impairment of affinity maturation in the 1b memory pool in suppressed mice must be a result of a diminished capacity for support of the IgD^+ to IgD^- transition rather than of removal or inactivation of either memory population. This conclusion suggests that 1b Th, the allotype-specific Th depleted from allotype-suppressed mice by the suppressor T cell (Ts), may be required to help the differentiation of 1b memory cells from IgD^+ to IgD^-.

The appearance of an IgD^- 1b memory population in suppressed mice that generally appears to be completely depleted for 1b Th can be explained by the following observations, which indicate that a temporary low-level release from suppression routinely occurs when suppressed mice are primed: Tested 1 wk after priming, suppressed mice frequently show small amounts of circulating 1b and almost always show some 1b anti-DNP antibody. The 1b anti-DNP levels at this time never reach >10% of the levels in similarly primed nonsuppressed mice and decay thereafter (data not shown). Thus it appears that priming temporarily interferes with the balance between Ts and Th and thereby allows expression of sufficient 1b Th activity to support some 1b memory cell differentiation from IgD^+ to IgD^- at the same time as it allows some differentiation of 1b afc.

This hypothesis is conservative in that it presumes a single 1b-specific Th that supports both 1b production and 1b memory transition. Alternatively, the memory transition could be specifically regulated by a second 1b Th (also depleted by Ts) or by some other 1b-specific mechanism impaired in allotype suppressed mice.

Discussion

The identification of persistent and coexistent memory B cell populations at two successive stages of differentiation, and the demonstration that high-affinity responses for the stimulating antigen are produced mainly by the more-mature population, offers a new perspective on the role of affinity maturation in humoral immunity. These findings suggest that the immune system in higher animals has evolved mechanisms that permit affinity-dependent selection to proceed within one memory population (i.e., the more mature) without interfering with the maintenance of a second (early) memory population that contains a lower affinity and hence more-diversely reactive precursor set. Therefore, the system seems adapted to enable generation of highly specific memory for the priming antigen and simultaneous retention of less-specific memory potentially capable of giving rise to high-affinity responses to subsequently encountered antigens that are related to, but different from, the original antigen.

Furthermore, because (as we have shown) the relative proportions of early and mature memory generated depend on priming conditions such as the adjuvant used and the carrier molecule on which the antigen is presented, our findings suggest that the immune system has developed regulatory mechanisms that allow maximum flexibility in determining how much of the initial response will be committed to immediate high-affinity response to the stimulating antigen and how much will be held in reserve for response to subsequent and potentially different (although related) antigens. Thus, the staged differentiation of memory B cells can be viewed as an evolutionary solution for the need to maintain a broad set of response options and yet

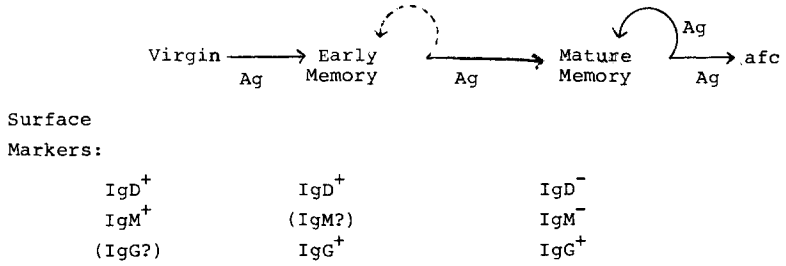

FIG. 3. Antigen (Ag)-dependent (memory) B cell differentiation pathway for IgG afc. Curved arrows indicate expansion (self renewal) of memory pools. The assignment of surface Ig markers other than IgD are discussed elsewhere.[2]

allow the narrowing of the response so that a high degree of specificity can be obtained when necessary.

The broad outlines of this developmental pathway are now beginning to emerge (Fig. 3). The first (early) memory population carries surface IgD and arises either by direct expansion or by differentiation and expansion of antigen-reactive virgin B cells. In either case, the generation of this population must, by definition, require an antigen-driven selection of cells whose receptors bind enough antigen to be triggered to differentiate and/or expand. Nevertheless, there appears to be relatively little selective expansion of higher- vs. lower-affinity cells during this process because, as we have shown, adoptive primary (virgin B) and early memory (IgD^+) responses differ in magnitude but approximate each other with respect to affinity. This may be because selective expansion in the adoptive primary may occur too rapidly to be observed in recipients supplemented with carrier-primed T cells. At present we can only state that the first memory transition (from virgin to IgD^+) appears to be relatively affinity independent in the sense that the affinity profile of the responding virgin population is similar to the profile of the progeny memory population.

The second memory transition (from IgD^+ to IgD^-) contrasts sharply in that it results in the development of a memory population that clearly gives rise to substantially higher-affinity responses than the average of the population from which it was drawn. Several lines of evidence, however, suggest that the actual cellular differentiation from IgD^+ to IgD^- in this transition also occurs in an essentially affinity-independent manner. Residual IgD^+ memory populations, for example, do not appear to be progressively depleted for precursors of high-affinity responses as the transition to IgD^- memory proceeds. The IgD^+ population, instead, appears to maintain the full range of response options present initially, even when the transition to IgD^- is nearly complete and relatively little IgD^+ memory remains.

The demonstration that IgD^+ memory populations disappear entirely during the memory transition also argues against selective differentiation of high-affinity precursors from IgD^+ to IgD^-, because low-affinity precursors would then be expected to accumulate and remain indefinitely as an IgD^+ population incapable of making the transition to mature memory. Thus it is likely that both lower- and higher-affinity precursors in the IgD^+ population have about the same probability of differentiating to IgD^-. The increased average affinity of the IgD^- memory population, therefore, appears solely to be a result of selective expansion of higher-affinity cells that have already differentiated and lost their IgD surface receptors.

Curiously, these arguments put us in the paradoxical position of having made a reasonably good case for what appears to be a highly unreasonable suggestion, i.e., that IgD^+ memory cells differentiate to IgD^- essentially randomly with respect to receptor affinity in an environment where their progeny IgD^- memory cells are actively being selected, apparently by limited antigen concentrations, to constitute a predominantly high-affinity memory population. The operation of such a system would appear to require either the unlikely maintenance of separate antigen depots at different concentration levels for each of the memory populations or the existence of an even less-likely mechanism for increasing the affinity of IgD^- receptors (e.g., by somatic mutation) during the transition from IgD^+ to IgD^-. Consideration of the antigen-binding capacity of memory cells as a function of both receptor affinity and receptor density, however, offers a more conventional solution to this problem and, at the same time, suggests a logical definition of the long-elusive role played by surface IgD receptors in the immune response.

The observed differences with respect to affinity maturation in early and late memory populations are predictable on the basis of the relative contributions made by IgD and IgG surface receptors to the (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.) The well-known difficulty in detecting IgG receptors, however, suggests that they are much more sparsely represented on the cell surface than are IgD receptors. This means that cells that lose their IgD receptors during differentiation from early to mature memory would be expected to have greatly reduced antigen binding capacity even though the specificity and affinity of the remaining (IgG) receptors remains constant. This reduction is tantamount to specifically reducing the antigen concentration for cells in the mature IgD^- memory population, because the probability that a cell with IgG receptors of a given affinity will capture antigen will be considerably decreased once its IgD receptors are lost. Thus, an antigen concentration high enough to allow the differentiations and expansions of IgD^+ populations to proceed relatively independently of receptor affinity can constitute a selective threshold that allows only higher-affinity IgD^- (IgG^+) memory cells to expand.

The above hypothesis is conservative in that it sees IgD receptors as functionally equivalent to other Ig receptors on B cells. Our data are also consistent, however, with the idea that IgD receptors function as more-efficient receptors for cell triggering. If so, then their presence would tend to maintain the IgD^+ memory population at even lower average affinity than might be expected merely from receptor-density considerations. Thus, our arguments concerning the role of IgD would be strengthened by the ad hoc assumption that IgD receptors are functionally adapted to increase sensitivity to limiting antigen concentrations.

IgD receptors, then, can be viewed as playing two related roles in memory development. First, they serve to extend the lower boundary of the affinity range of virgin and early memory B cell populations and thus broaden the potential diversity of responses by these populations. This means that early memory populations, so long as they persist, will retain a cross-reactive capability that tends to be lost in mature memory populations that have higher combining affinities for stimulating antigens. Second, because early memory populations constitute a reservoir from which IgD^+ memory populations are drawn, the ability of IgD receptors to maintain parity among

cells with broadly different receptor affinities allows partial depletion of the IgD^+ pool without sacrifice of the full range of response diversity in the residual IgD^+ memory population. The advantages offered by these functions could well be expected to account for the evolutionary development of IgD receptors and the regulatory capabilities that support operation of the system.

The emergence of a probable role for IgD receptors as modifiers of the potential for affinity maturation in early vs. late memory populations constitutes (for us) a rather surprising outcome of this series of studies. Nevertheless, the hypothesis to which our data leads us appears quite viable. Other investigators have suggested that IgD receptors are important for preventing tolerance induction (14) or for stimulating idiotype suppression (15). The role we suggest for IgD does not negate these hypotheses because there is no a priori reason why IgD receptors cannot serve several purposes. Similarly, these other hypotheses do not exclude a major role for IgD in affinity maturation. Thus, IgD receptors may serve several purposes, not the least of which is the support of low-affinity (early) memory populations in a selective environment.

The regulatory principles inherent in this view of B cell memory development also remain in accord with previous concepts but add significant new dimensions to these concepts. Our data, for example, are fully consistent with the idea (1) that antigen-driven selection of higher-affinity clones is responsible for the increased response affinities obtained from memory populations. Similarly, our data confirm the previously reported (4) requirement for T cell help in developing selectively expanded B cell populations that give rise to higher-affinity responses. But the evidence presented that indicates that T cells may regulate the extent of transition from early to mature memory introduces a novel level of control of memory development and affinity maturation. These findings suggest the existence of regulatory interactions specifically responsible for determining the balance between conservation of a broadly reactive precursor reservoir and the development and expression of high-affinity memory.

Current information, unfortunately, is too sparse to allow a definitive description of these regulatory interactions. We have implicated two types of Th in memory regulation: CTh and Ig Th. We have shown (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.) that the CTh are required at least for expansion of mature (IgD^-) cells, whereas Ig Th appear to be required at least for the differentiation of early (IgD^+) to mature (IgD^-) memory. This suggests an attractive model in which each type of Th is specialized for a given function (i.e., for helping either differentiation or expansion); however, because the experimental conditions used did not allow exclusive definition of help interactions, our data are consistent either with a division of labor hypothesis or with a hypothesis that sees CTh and Ig Th as jointly required for differentiation and/or expansion.

It is also difficult, at this time, to definitively assign Ig Th as helpers of the IgD^+ to IgD^- differentiation step. The data we have presented on this point are derived from *in situ* priming studies with allotype-suppressed mice rather than memory cell isolation and transfer experiments such as those that showed that CTh are required for IgD^- memory expansion (Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. Memory B cells at successive stages of differentiation: expression of surface IgD and capacity for self renewal. Manuscript in preparation.). The IgD^+ to IgD^- differentia-

tion is clearly hampered in the 1b memory population in (1b) suppressed mice. This suggests (but does not prove) that this differentiation fails in the absence of an Ig Th population dedicated to helping 1b B cells (i.e., 1b Th). These Th, as we have shown previously (11, 16, 17), are required for 1b production and are specifically depleted by allotype Ts in allotype-suppressed mice; but whether the lack of 1b Th or other conditions in the suppressed mice prevent affinity maturation has yet to be determined.

Our studies with primed normal mice have also shown that genetic elements and priming conditions such as the adjuvant used and the carrier protein on which the priming hapten is presented exert strong influences in the determination of the proportion of the memory population that is maintained in the less-mature (IgD^+) state. The importance of adjuvant and carrier again suggests that T cells regulate the IgD^+ and IgD^- memory transition in addition to regulating IgD^- expansion.

In sum, the studies presented here introduce the idea that IgD receptors play a crucial role in supporting an early appearing low-affinity memory population under conditions where its more mature (IgD^-) progeny population is actively undergoing affinity maturation. The prolonged coexistence of these two memory populations under some, but not all, conditions of antigenic stimulation, we suggest, indicates that the immune system has evolved regulatory mechanisms that optimize the balance between development of highly specific (high-affinity) responses and the maintenance of broadly reactive (low-affinity) memory. This potential for optimization would appear to adapt the animal to deal with the variety of naturally encountered antigens and therefore to justify the evolution of such highly complex mechanisms of memory development and regulation.

Summary

The following evidence, mainly presented here, suggests that IgD receptors play a crucial role in determining the potential for affinity maturation in memory B cell populations. IgD receptors are present on the first memory B cells to appear after priming. These memory cells give rise to more-mature memory cells that have lost their IgD receptors. The proportions of early (IgD^+) and mature (IgD^-) memory cells found in individual donors vary with time, priming conditions, and the availability of T cell help, and both populations frequently coexist for long periods of time.

IgD^+ and IgD^- memory cells carry IgG receptors and give rise to IgG responses with identical isotype representation in adoptive recipients. IgD^+ memory cells, however, always give rise to predominantly low-affinity antibody responses, whereas IgD^- memory cells consistently generate responses of substantially higher average affinity. This affinity differential is maintained between early and mature memory populations in the same donor and does not appear to be a result of selective differentiation of higher-affinity IgD^+ memory cells into the IgD^- memory pool. Thus, the selective forces responsible for affinity maturation appear to operate mainly in mature memory cell populations that have already lost IgD receptors; or, stated conversely, little or no selection towards high-affinity memory appears to occur among memory cells that retain IgD receptors.

In discussing these findings, we suggest that the IgD receptors themselves are responsible for maintaining early memory populations at a lower average affinity than IgD^- populations in the same animal. The IgD receptors, we argue, serve to

increase the antigen-binding capacity of lower-affinity memory cells so that these cells can survive, expand, and differentiate (to IgD^-) at antigen concentrations that select against expansion of low-affinity memory cells no longer carrying IgD receptors. Thus, when antigen is limiting, IgD^- memory populations will be selectively expanded to higher average affinities, whereas coexisting IgD^+ populations will retain their initial affinity profile.

This hypothesis suggests that mechanisms that regulate expression and loss of IgD receptors are central to the adaptability of the immune system in its response to invading pathogens. Two related roles can be envisioned for the IgD receptors in this regard. First, they extend the lower boundary of the affinity range of early memory cell populations induced by a given antigenic stimulus and therefore broaden the diversity of responses obtainable from these populations. Secondly, they support the persistance of low-affinity memory populations under conditions where antigen becomes limiting and eventually disappears. These persisting populations then serve as a diversely reactive reservoir from which mature memory populations can be drawn with higher affinities either for the original antigen or, more importantly, for related antigens that the animal may subsequently encounter. Thus the existence of IgD receptors on early memory cells maintains the full range of response diversity despite ongoing selective expansion of (mature) memory populations to produce antibodies with high combining affinities for individual antigens. The flexibility inherent in such an organizational system, we believe, could be expected to account for the evolutionary development of IgD receptors and the regulatory capabilities that support operation of the system.

We wish to thank Ms. Sandy Scaling and Mr. Timothy Gadus for their dedicated and innovative technical assistance in the completion of this project. Dr. Jeffrey Ledbetter, Mr. Vernon Oi, Dr. Kyoko Hayakawa (Tokyo University, Tokyo, Japan), and Mr. Theta Tsu made significant contributions to the development of the radioimmune assays used here to measure the avidities of anti-DNP antibody. Mr. Eugene Filson and Mr. Wayne Moore, by respectively operating and programming the FACS equipment, also provided invaluable assistance, as did Ms. Jean Anderson in preparation of this manuscript. To these and all the other technical and research personnel in our laboratory who maintain the complex reagents and equipment necessary for this extraordinarily demanding study, we extend our deeply felt gratitude.

Received for publication 19 November 1979 and in revised form 30 January 1980.

References

1. Paul, W. E., G. W. Siskind, B. Benacerraf, and Z. Ovary. 1967. Secondary antibody responses in hapten systems: cell population selection by antigen. *J. Immunol.* **99:**760.
2. Black, S. J., W. van der Loo, M. R. Loken, and L. A. Herzenberg. 1978. Expression of IgD by murine lymphocytes. Loss of surface IgD indicates maturation of memory B cells. *J. Exp. Med.* **147:**984.
3. Black, S. J., T. Tokuhisa, and L. A. Herzenberg. 1978. Regulation of memory B cell differentiation. *In* B Lymphocytes in the Immune Response. M. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier North-Holland, Inc., New York. 145.
4. Gershon, R. K., and W. E. Paul. 1971. Effect of thymus derived lymphocytes on the amount and affinity of anti-hapten antibody. *J. Immunol.* **106:**872.
5. Okumura, K., C. M. Metzler, T. T. Tsu, L. A. Herzenberg, and L. A. Herzenberg. 1976. Two stages of B-cell memory development with different T-cell requirements. *J. Exp. Med.* **144:**345.

6. Herzenberg, L. A., and L. A. Herzenberg. 1973. Short-term and chronic allotype suppression in mice. *Contemp. Top. Immunobiol.* **3:**41.
7. Herzenberg, L. A., and L. A. Herzenberg. 1978. Analysis and separation using the fluorescence activated cell sorter (FACS). *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford. 3rd edition. 22.1–22.21.
8. Tsu, T. T., and L. A. Herzenberg. 1980. Solid-phase radioimmune assays (RIA). *In* Selected Methods in Cellular Immunology. B. B. Mishell and S. M. Shiigi, editors. W. H. Freeman & Co., Publishers, San Francisco. 373.
9. Pecht, I., D. Givol, and M. Sela. 1972. Dynamics of hapten-antibody interaction. Studies on a myeloma protein with anti-2,4-dinitrophenyl specificity. *Mol. Biol. Biochem. Biophys.* **68:**241.
10. Johnston, M. F., B. G. Barisas, and J. M. Sturtevant. 1974. Thermodynamics of hapten binding to MOPC-315 and MOPC-460 mouse myeloma proteins. *Biochemistry.* **13:**390.
11. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F.-W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
12. Woodland, R., and H. Cantor. 1978. Isiotype-specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8:**600.
13. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte interactions. II. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8:**853.
14. Kettman, J. R., J. C. Cambier, J. W. Uhr, F. Ligler, and E. S. Vitetta. 1979. The role of receptor IgM and IgD in determining triggering and induction of tolerance in murine B cells. *Immunol. Rev.* **43:**69.
15. Bourgois, A. E., E. R. Abney, and M. E. Parkhouse. 1977. Mouse immunoglobulin receptors on lymphocytes: identification of IgM and IgD molecules by tryptic cleavage and a postulated role for surface IgD. *Eur. J. Immunol.* **7:**210.
16. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. *Transplant. Rev.* **27:**57.
17. Black, S. J., and L. A. Herzenberg. 1979. B-cell influences on the induction of allotype suppressor T cells. *J. Exp. Med.* **150:**174.