2. Materials and methods

2.1. Introductory remarks

Most of the materials and methods used for studies presented here are described in detail elsewhere*. The following briefly summarizes these methods and adds others unique to studies in this publication.

2.2. Mice

(SJL/J x BALB/cN)F_1 spleen cell hybrid donors and BALB/cN recipient mice raised in our colony were used for this study. Allotype-suppressed donors used for the Ig-1b separation were (SJL x BALB/c)F_1 mice exposed perinatally to maternal (BALB/c) anti-Ig-1b. Donors were generally over six months of age and always tested for Ig-1b just prior to transfer. Only donors showing no serum Ig-1b detectable by immunodiffusion (< 0.01 mg/ml) were used.

2.3. Priming

Donors were primed with 100 μg 2,4-dinitrophenyl (DNP) keyhole limpet hemocyanin (KLH) on alum (hapten priming) or with 100 μg KLH on alum (carrier priming). Both antigens were injected i.p. with 2 x 10^9 of *Bordetella pertussis* vaccine (kindly supplied by Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y.). Mice were used as donors a minimum of two months after priming.

2.4. Adoptive transfer and plaquing

Spleen cells from various donors were suspended in minimum essential medium (MEM) and mixed at appropriate doses just prior to i.v. injection into BALB/c recipients irradiated (600 r) 18 h previously. Recipients were challenged at time of transfer with 10 μg aqueous DNP-KLH and sacrificed 7 days later for determination of DNP-PFC in spleens. DNP-PFC were measured in Cunningham chambers. Indirect DNP-PFC were measured by determining the increase in DNP-PFC in chambers containing the appropriate facilitating antiserum over the response in chambers with no facilitating antisera (direct DNP-PFC). Results are expressed as DNP-PFC/10^6 recipient spleen cells. Spleen size in adoptive recipients did not vary substantially.

2.5. Preparation of antisera for plaquing and staining of IgG-bearing cells

Total IgG DNP-PFC were developed with a rabbit antiserum reactive with all IgG classes. Specific anti-allotype antisera were used to develop Ig-1a and Ig-1b DNP-PFC. Specific anti-class antisera were used to develop IgG_1 and IgG_{2b} DNP-PFC. For the staining of Ig-1b, IgG_1-bearing cells, the same antisera used to develop PFC, were used. These antisera were rendered specific by appropriate absorbtion with insoluble immunoabsorbants. The starting serum for anti-Ig-1a was produced by immunizing a goat with the Ig-1a myeloma protein 5563. This serum was absorbed by sequentially passing through Sepharose 4B columns to which Ig^b (C57BL/10) immunoglobulins and Ig-4a (S-8) myeloma proteins were covalently bound. The starting serum for the anti-Ig-1b (kindly supplied by Dr. John Coe, Rocky Mountain National Laboratory) was produced by immunizing a rabbit with C57BL/6 (Ig^b) globulins. This was absorbed by sequentially passing through columns containing BALB/c-Ig, Ig-1a (RPC-5 myeloma protein) and Ig-4b (MOPC-245T myeloma protein) coupled to Sepharose 4B. The antiserum was further purified by passing it through Ig^b (C57BL/10) immunoglobulin-coupled Sepharose 4B column and eluting off the specifically bound anti-Ig-1b [7]. For IgG_1 DNP-PFC and staining of IgG_1-bearing cells, an anti-IgG_1 was produced by immunizing a goat with an Fc fragment from a mouse IgG_1 (MOPC-21 myeloma protein). This was absorbed by sequentially passing through columns containing IgG_{2a} (RPC-5 myeloma protein) and IgG_{2b} (MPC-11 myeloma protein) coupled to Sepharose 4B.

For the staining of IgG_{2a} an anti-IgG_{2a} was produced by immunizing a goat with an Fc fragment of Ig-1a (RPC-5 myeloma protein). This was absorbed by passing through a column containing Ig-4a (S-8 myeloma protein) coupled to Sepharose 4B. The antiserum was further purified by passing it through a Sepharose column to which RPC-5 myeloma proteins were covalently bound and eluting off the specifically bound anti-Ig-1a. All sera were tested for specificity by radioimmunoassay. Immediately before staining, the antisera were centrifuged at 100 000 x g for 30 min to remove any aggregates.

2.6. Immunofluorescent staining

Before staining of spleen cell suspensions or T-depleted spleen cell suspensions (B cells), erythrocytes were lysed by incubating the cells for two min at 4 °C in Gey's balanced salt solution in which the NaCl was replaced with an equimolar concentration of NH_4Cl [8].

Cells were stained by two-step staining. Spleen cells (10 x 10^6) were centrifuged in conical tubes and resuspended in 0.1 ml of anti-mouse antisera. After 30 min incubation at 4 °C the cells were underlayed with heat-inactivated fetal calf serum (FCS) and centrifuged. Cells were then washed once with Dulbecco's phosphate buffered saline (PBS) with 5 % FCS.

Cells were resuspended in 0.1 ml fluorescein-conjugated goat anti-rabbit IgG (^{F}goat anti-rabbit IgG) or rabbit anti-goat IgG (^{F}rabbit anti-goat Ig). After 30 min incubation at 4 °C the cells were underlayed with FCS and washed with Dulbecco's PBS with 5 % FCS.

2.7. FACS isolation of IgG-bearing cells

The FACS isolation used for these experiments allows separation of cells according to amount of bound fluorescence, amount of low-angle light scattering (size) or selected combination of these two parameters [9]. A two-stage separation protocol was used to obtain efficient sorting of the small number of Ig-1b-bearing cells present (< 1 % of splenic B cells). On the first pass, the brightest 10 % of cells and the dullest 85 % were collected. On the second pass the brightest 10 % of the first-pass bright cells were taken. In the IgG_1 separa-

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Demonstration that IgG memory is carried by IgG-bearing cells*

Memory B cells which give rise to IgG antibody-producing cells were generally assumed to be IgG-bearing cells. However, recent studies indicating that very few IgG-bearing cells exist in lymphoid tissue brought this assumption into question. In this study, we examined directly the question whether IgG-bearing cells contain functional precursors of IgG antibody-producing cells. Using the adoptive secondary immune response, we demonstrated that Ig-1b-bearing cells, isolated with the fluorescence-activated cell sorter (FACS), are the functional precursors of Ig-1b-producing cells. Further, we have enriched IgG_2 and IgG_1 memory B cells using the FACS. The results show that IgG_2-bearing cells are the functional precursors of the IgG_2 antibody-producing cells. Likewise, the IgG_1-bearing cells are the functional precursors of IgG_1 antibody-producing cells. Thus, IgG memory cells have surface IgG which indicates the class and allotype commitment of the memory cell and its progeny antibody-forming cells.

1. Introduction

Until recently, the memory B cells which give rise to IgG antibody-forming cells (AFC) were generally assumed to be IgG-bearing cells. The arguments in favor of this hypothesis, aside from the reasonability of expecting that the surface Ig on B cells indicates their commitment with respect to immunoglobulin class and allotype, included studies which show that IgG-bearing cells are found in lymphoid tissue [1, 2]; that the appearance of IgG-bearing cells is correlated developmentally with the ability to make IgG memory responses [3, 4]; that *in vivo* or *in vitro* exposure of immunocompetent populations to antibody reactive with a given IgG class specifically prevents production of that IgG class [3, 4]; and that pretreatment of memory cells with anti-IgG prevents the memory cells from adhering to the priming antigen coupled to an insoluble absorbant [5]. Studies such as these, although admittedly indirect, made it appear likely that IgG memory cells carry surface IgG.

Vitetta and Uhr, however, question the validity of this conclusion [6] because of the small numbers of IgG-bearing lymphocytes present in lymphoid tissues. They state that as the specificity of the methodology for detection of IgG-bearing lymphocytes has improved, the number of these cells found in spleen or peripheral blood has decreased to the order of 1 % of total cells, and suggest this number may well be zero if other methodological artifacts can be eliminated. Even if the 1 % figure does represent a real population of cells with IgG surface receptors, they argue, such a population would be too small to serve as the repository for IgG memory. Therefore, these authors propose an alternate model of B cell differentiation in which memory cells carry IgD (delta) surface receptors rather than IgG.

In the studies presented here, we take a more direct approach to the question of which B cells carry memory. We use the fluorescence-activated cell sorter (FACS) to isolate populations of hapten-primed splenic B cells bearing each of three types of IgG molecules (IgG_1, IgG_2 and Ig-1b, an allotype on IgG_{2a} globulins) and then test both the isolated populations and residual populations from which the isolated cells were drawn for the ability to transfer memory in an adoptive secondary hapten-carrier assay. As we will show, the results of these studies demonstrate that B cell memory is carried by IgG-bearing lymphocytes despite the small number of these cells found in spleen. Furthermore, we will show that the surface IgG indicates the class and allotype commitment of the memory cell and its progeny AFC and therefore is most likely endogenously synthesized by the memory cells.

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Abbreviations: **FACS:** Fluorescence-activated cell sorter **DNP:** 2,4-Dinitrophenyl **KLH:** Keyhole limpet hemocyanin **PFC:** Plaque-forming cell **AFC:** Antibody-forming cell **MEM:** Minimum essential medium **FCS:** Fetal calf serum **PBS:** Phosphate buffered saline

Table 2. Presence of DNP memory in population of Ig-1b-bearing cells from chronically suppressed SJL x BALB/c hybrids

DNP-KLH-primed spleen cells transferred (x 10^6)			Indirect DNP-PFC[a]			
Description[b]	Total number of cells	Number of Ig-1b[d]-bearing cells	Total IgG	Ig-1b	Ig-1a	IgG_1
T-depleted, stained for Ig-1b	5	~0.04	2970	335	356	980
Dullest 85 %[c]	5	< 0.0005	2620	31	310	870
Brightest 1 %[c]	0.05	0.045	185	198	0	0

a) Indirect DNP-PFC/10^6 recipient spleen cells. Direct DNP-PFC (< 50) subtracted.
b) T-depleted cells were prepared by treating spleen cells from DNP-KLH-primed mice with anti-Thy-1 plus complement. Fluorescent Ig-1b cells were obtained by staining the T-depleted cell suspension first with rabbit anti-Ig-1b, then with ^{F}goat anti-rabbit IgG. After staining, the cells were passed through the FACS to obtain separated fractions. All adoptive transfer recipients of T-depleted spleen cells, or separated fractions were given 10^7 KLH-primed spleen cells (helper T cells) from nonsuppressed (SJL x BALB/c)F_1 donors.
c) Percentage of T-depleted B cell suspension after anti-Thy-1 treatment.
d) Number of Ig-1b-bearing cells as determined by fluorescence microscopy.

3.3. IgG_2-bearing cells carry IgG_2 memory

Separation and testing of IgG_2-bearing cells yielded results similar to the separation of Ig-1b-bearing cells: the isolated cells carried only IgG_2 memory while the depleted population carried memory only for other classes of IgG antibody (see Table 3).

Following a protocol similar to the Ig-1b separation (see Section 2.7.), the brightest 4 % and dullest 80 % of B cells indirectly stained with a goat anti-IgG_2 reagent were supplemented with carrier-primed T cells and tested in an adoptive secondary assay. B cells from (SJL x BALB/c) hybrids primed with DNP-KLH were used as starting material for the separation. As the data in Table 3 show, the isolated cells gave mainly IgG_{2a} and IgG_{2b} DNP-PFC. The sum of these two IgG_2 responses nearly equaled the "total IgG" response detected with a polyvalent anti-IgG serum. Conversely, the depleted (non-IgG_2-bearing cells) showed almost no IgG_2 response over background, whereas the "total IgG" response and the IgG_1 response were essentially the same as with unseparated B cells.

3.4. IgG_1-bearing cells carry IgG_1 memory

The results of the IgG_1 separation (done as detailed in Section 2.7.) differ somewhat from the preceding two separations. The IgG_1-bearing cells still give rise only to IgG_1 DNP-PFC, but the depleted population retained roughly half of the IgG_1 memory detectable in the unseparated population (see Table 4).

The protocol used for this separation was similar to the preceding two, except that the anti-IgG_1 reagent did not stain the cells brightly enough to allow visibility with the fluorescence microscope or to allow resolution into a discrete population by the quantitative fluorescence measurement with the FACS.

4. Discussion

The evidence presented here shows that B cell memory, as defined by the ability to transfer an adoptive secondary anti-hapten response, is carried by IgG-bearing cells. These findings are of considerable importance in establishing the sequence of B cell differentiational events from virgin precursor to AFC [11]. Discussion of IgG-bearing cells within this framework, however, first requires a more detailed consideration of the data upon which we base our conclusions.

Table 3. Presence of DNP memory in IgG_2-bearing cells separated by FACS

(SJL x BALB/c)F_1 spleen cells transferred (x 10^6)					Indirect DNP-PFC[a]			
DNP-KLH-primed donors			KLH-primed donors					
Description[b]	Number of cells	Number of[d] IgG_2-bearing cells	Number of cells	IgG_{2a}	IgG_{2b}	IgG_1	Total IgG
Untreated	6			1190	281	3150	5030
T-depleted, stained for IgG_2	3			58	17	304	415
T-depleted, stained for IgG_2	3	~0.12	10	957	195	2460	4010
Dullest 80 %[c]	3	< 0.0005	10	40	26	1920	3220
Brightest 4 %[c]	0.12	0.10	10	532	77	76	721

a) Indirect DNP-PFC/10^6 recipient spleen cells. Direct DNP-PFC (< 120) subtracted. IgG_{2a} DNP-PFC are calculated by adding Ig-1a and Ig-1b DNP-PFC.
b) T-depleted cells were prepared by treating spleen cells with anti-Thy-1 plus complement. Fluorescent IgG_2 cells were obtained by staining the T-depleted cell suspension first with goat anti-IgG_2, then with ^{F}rabbit anti-goat IgG. After staining, the cells were passed through the FACS to obtain separated fractions.
c) Percentage of T-depleted B cell suspension after anti-Thy-1 treatment.
d) Number of Ig-1b-bearing cells as determined by fluorescence microscope.

tion 20 % of first-pass and 50 % of second-pass cells were taken. In the IgG_2 separation, 10 % of first-pass and 40 % of second-pass cells were taken. Dead cells (< 10 %) were removed during both separations by accepting for fluorescence sorting only those cells with light-scatter properties characteristic of live cells [9].

2.8. Microscope counting of fluorescent Ig-1b-bearing cells

Since less than 1 % of spleen cells from heterozygous mice are Ig-1b-bearing cells, direct microscope counting of spleen cell smears is difficult and relatively inaccurate. Therefore, spleen cell suspensions stained as above were passed through the FACS. The brightest 5 % and 10 % were isolated in a small glass cylinder cemented to a microscope slide. After a sufficient number of cells were collected, the slide was centrifuged for 10 min in a cytocentrifuge (Shandon Scientific Co. Ltd., London, England), the fluid drained from the well and the well broken off the slide. Slides were fixed in 95 % ethanol for 15 min and mounted in phosphate buffered glycerol pH 8.5. The percentage of fluorescent cells corrected for the enrichment by FACS passage, was determined with fluorescence microscope. This procedure yielded an accurate estimate of the percentage of Ig-1b-bearing cells in spleens.

3. Results

3.1. Staining of IgG-bearing cells in spleen

Data in Table 1 shows the percentage of IgG-bearing cells detectable in spleen under the fluorescent microscope after fluorescent staining with the three reagents used for separations in these studies. Approximately 2 % of spleen cells are visibly stained with anti-IgG_2 reagent and approximately 0.5 % of cells are stained with anti-Ig-1b. Since the brightness of anti-Ig-1b-stained cells is less than that of anti-IgG_2-stained cells, the number of Ig-1b-bearing cells visible by microscope may be less than the actual number of Ig-1b-bearing cells.

Only a few IgG_1-bearing cells were made clearly fluorescent by staining with the IgG_1 reagent. Very dimly stained cells with patchy fluorescence were visible under the fluorescence microscope, but differentiating between these and nonstained cells was too difficult to allow a valid count. Separation data (presented later) indicate that the IgG_1-bearing cells were stained with the IgG_1 reagent, and therefore point out the difficulties in using microscope counting to estimate the number of cells carrying a particular surface determinant.

Table 1. Staining of IgG-bearing cells in spleen

First step[a] staining reagent	Total spleen cells stained visible by fluorescence microscope	Cells[c] isolated by FACS
	(%)	(%)
Goat anti-IgG_1	1	10
Goat anti-IgG_2	2.0	4
Rabbit anti-Ig-1b[b]	0.6	1

a) ^{F}Goat anti-rabbit Ig or ^{F}rabbit anti-goat IgG was used for second-step reagent.
b) Ig-1b is an allotype of IgG_{2a}. Donors used were Ig-1b/Ig-1a heterozygotes (SJL x BALB/c).
c) Number represents percentage cells isolated from T-depleted spleen cells.

For separation, as indicated in Table 1, the number of cells taken into the IgG_2 and Ig-1b-bearing cell fractions with the FACS was based on the number of visibly fluorescent cells. An arbitrary 10 % of the brightest IgG_1-stained cells in T-depleted cell suspensions detectable with the FACS were taken as IgG_1-bearing cells. In all three cases, the "next brightest" 10 % of cells was discarded to avoid contaminating the depleted fraction with dully staining IgG-bearing cells.

3.2. Ig-1b-bearing cells carry Ig-1b memory

To isolate Ig-1b-bearing cells, T-depleted (anti-Thy-1-treated) spleen cell suspensions from hapten-primed donors were incubated first with an absorbed rabbit antiserum specific for Ig-1b. Donors were allotype-suppressed [10] and had no detectable serum Ig-1b. T depletion removed all allotype suppressor T cells.

After indirect staining for Ig-1b, the cells were FACS-sorted by the double-pass procedure (see Section 2.7.). In the first pass, the brightest 10 % and the dullest 85 % were saved. In the second pass, the brightest 10 % of the cells was further separated to obtain a cell fraction which contained the brightest 10 % of cells in the fraction, thus the brightest 1 % of the original anti-Thy-1-treated spleen suspension. All viable Ig-1b-bearing cells visible by fluorescence microscope were in this fraction. These comprise approximately 0.5 % of the original spleen cell suspension before T depletion.

The two fractions of separated cells, *i.e.* the brightest 1 % (enriched for Ig-1b-bearing cells) and the dullest 85 % (depleted for Ig-1b-bearing cells), were supplemented with KLH-primed spleen cells from normal (nonsuppressed) donors to provide an excess of T helper activity, and tested for memory B cell activity in the adoptive secondary response to DNP-KLH. Dosages of transferred cells were adjusted so that the number of isolated bright or dull cells transferred would be equal to the number of those cells transferred in the original unseparated suspension.

The data in Table 2 show that the isolated fraction enriched for Ig-1b-bearing cells, comprising only 1 % of the unseparated cells, contained roughly two-thirds of the Ig-1b memory cells found in the unseparated spleen suspension. This fraction, however, did not contain memory cells for DNP-PFC-producing antibody of any other immunoglobulin class. In sharp contrast, the fraction depleted for Ig-1b-bearing cells (dullest 85 %) contained less than 10 % of the Ig-1b memory cells in the unseparated suspension. Instead it contained essentially all of the other IgG memory cells.

Thus, Ig-1b-bearing cells carry Ig-1b memory and are committed exclusively to the production of Ig-1b, at least within the confines of the adoptive secondary response. Furthermore, non-Ig-1b-bearing cells do not carry Ig-1b memory, although these cells do contain among them memory B cells which give rise to all other IgG antibody-forming cells. The quantitative limits of this conclusion are discussed in Section 4.

phocytes of the sort he has previously shown carry most IgG memory.

We have presented evidence elsewhere which suggests that the surface IgG on memory cells may also provide a specific recognition site for helper T cells which allows selective help for B cells committed to a given allotype or class. In SJL x BALB/c hybrids such as those used for studies presented here, the helper T cells which help Ig-1a or IgG_1 B cells do not appear to help Ig-1b B cells. While it is not clear whether the helper T cell recognizes the Ig-1b memory cell by virtue of its surface Ig-1b (although possibly by associated idiotypic specificities), a parsimonious hypothesis would again suggest this as a likely probability.

We have used three different Fc-specific antibody reagents to (fluorescent) stain cells for sorting with the FACS. In each case, IgG_1, IgG_2 and Ig-1b, the isolated stained cells tested in an adoptive secondary response, gave rise to AFC which produced the same Fc class of antibody detected on the memory cell surface by the staining reagent.

The starting population for the FACS isolation of the Ig-1b-bearing memory cells was a T-depleted (anti-Thy-1-treated) DNP-KLH-primed spleen cell suspension from Ig-1b allotype-suppressed mice. Spleen cells from suppressed mice were used because these mice have no detectable serum Ig-1b and therefore would not be expected to have any cytophilic antibody of this type. Anti-Thy-1-treated suspensions were used for several reasons although we have also isolated Ig-1b-bearing cells from spleen cell suspensions while T cells were present (Okumura and Herzenberg, unpublished observation). Anti-Thy-1 treatment of the starting population is preferable because it assures the removal of allotype suppressor T cells which suppress Ig-1b production. This allows all fractions to be tested in adoptive transfer for Ig-1b memory cells. T depletion prior to separation is also preferable because treatment then enriches for B cells and thus cuts about in half the time required for FACS separation of the required number of cells.

We have also shown elsewhere that B cell memory occurs in two stages*, the first of which occurs after priming, does not require mature T cells (*i.e.* is T-independent) and results in the establishment of a pool of memory B cells with low average avidity. The second stage, which occurs after repeated boosting and requires mature helper T cells, results in an increase in the average avidity, the memory cell pool. The data presented in this publication show that the first-stage memory cells bear IgG since a) the memory cells for all separations were taken after a single priming dose of antigen and b) the Ig-1b memory cells were taken from Ig-1b allotype-suppressed mice in which the Ig-1b memory pool cannot undergo the second stage transformation to higher avidity because the suppressor T cells in these mice specifically remove the helper T cell activity which helps Ig-1b B cells.

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Table 4. Presence of DNP memory in IgG_1-bearing cells separated by FACS

Description[b]	Spleen cells transferred ($\times 10^6$) DNP-primed donors Number of cells	KLH-primed donors Number of cells	Indirect DNP-PFC[a] IgG_1	IgG_{2a}	Total IgG
Unfractionated	5		3860	1090	6450
T-depleted, stained for IgG_1	2.5		133	24	244
T-depleted, stained for IgG	2.5	4	3220	874	5770
Dullest 80 %[c]	2.5	4	1340	938	3510
Brightest 10 %[c]	0.25	4	1320	<10	1460

a) Indirect DNP-PFC/10^6 recipient spleen cells. Direct DNP-PFC (< 140) subtracted. IgG_2 DNP-PFC are calculated by adding Ig-1a and Ig-1b DNP-PFC.

b) T-depleted cells were prepared by treating spleen cells with anti-Thy-1 plus complement. Fluorescent IgG_1 cells were obtained by staining the T-depleted cell suspension first with goat anti-IgG_1, then with Frabbit anti-goat IgG. After staining, the cells were passed through the FACS to obtain separated fractions.

c) Percentage of T-depleted B cell suspension after anti-Thy-1 treatment.

The Ig-1b-bearing cells in the B cell population were stained with a fluorescein-conjugated rabbit antiserum to C57BL/6 immunoglobulins rendered specific for Ig-1b by immunoabsorption. The serum was tested for specificity for Ig-1b by radioimmune assay; however, the ultimate test of its specificity is in these studies where it fails to stain memory cells other than those committed to give rise to Ig-1b-producing cells.

Less than 10 % of the Ig-1b memory cells demonstrable in the unseparated (starting) population is found in the Ig-1b-depleted (unstained) fraction. These memory cells could be either non-Ig-1b-bearing cells or Ig-1b-bearing cells improperly classified by the FACS. In any event, the 10 % figure represents the upper limit of non-Ig-1b-bearing cells in spleen which give rise to Ig-1b DNP-PFC by seven days after transfer since, as we have shown elsewhere (see footnote, Section 2.) the DNP-PFC response at a saturating helper T cell dose (such as that used in this experiment) is proportional to the B cell dose, hence to the number of memory cells transferred. Actually, the Ig-1b response mounted by this fraction may not be significantly above zero considering the background IgM response of equal magnitude which had to be subtracted from the observed anti-Ig-1b facilitated response (see Section 2.).

The isolated populations in IgG_1 and IgG_2 separations showed results similar to the Ig-1b separation. In all three cases the isolated population contained only those memory cells capable of giving rise to AFC producing the same IgG class and allotype as the surface IgG Fc determinants detected by the staining reagent. Thus, these separations indicate that the surface IgG Fc determinants on memory cells mark the IgG commitment of the cells and their progeny.

The results with the depleted residual populations support this conclusion, although the IgG_1-depleted population leaves some room for question. In the IgG_2 separation, as in the Ig-1b separation, the residual population was virtually completely depleted for memory cells able to give rise to the AFC producing the IgG found on the isolated cells, indicating that most, if not all, IgG_2 memory is carried by IgG_2-bearing cells. In the IgG_1 separation, however, substantial IgG_1 memory remained with the residual cells. This may be due to incomplete separation of IgG_1-bearing cells, either because the IgG_1 reagent used for staining was too weak to sufficiently demark the IgG_1-bearing cells from background fluorescence or because IgG_1-bearing cells comprise more than the top 5 % of cells stained with this reagent. This cut-off had to be chosen arbitrarily because of the weak staining. These technical considerations may explain the inability to isolate all IgG_1 memory cells in the IgG_1-bearing cell populations; however, it is also possible that unlike IgG_2 memory, IgG_1 memory is carried in part by IgG_1-bearing cells and in part by B cells with different (or no) surface immunoglobulin. Resolution of this point will require further study.

Establishing IgG-bearing cells as the major, if not unique, repository for B cell memory raises questions as to the role of the surface IgG. Strictly speaking, we have not proved that the Fc determinants detected by the staining reagents are integrated into whole IgG molecules; however, since Fc fragments have not been found in preparations of surface immunoglobulins precipitated by anti-IgG antibody, it is reasonable to assume that our reagents detect the Fc portion of IgG molecules. We also have not directly demonstrated that the surface IgG we detect is produced by the cells which we isolate; however, the direct correlation between surface IgG class and IgG class produced makes it highly unlikely that the surface IgG is exogenously acquired. Most likely, then, the surface IgG represents "sample" IgG molecules produced by memory cells and indicating the class and allotypic commitment of each cell.

These same IgG molecules would also, in a parsimonious hypothesis, be expected to indicate the commitment of the memory cells with respect to antigenic specificity. Julius et al. [12] have shown clearly that the specificity of antigen receptors on memory B cells indicates the specificity of the IgG antibody-forming cells to which these cells give rise. In further studies, we have shown [13] that the avidity of antigen receptors on the "virgin" B cells responsible for the adoptive (IgM) primary response determines the avidity of the antibody produced. These studies, taken together with the demonstration by Jones et al. [14] that in the rabbit, B cells carrying a given Fab allotype give rise to AFC producing the same allotype suggest strongly that antigen receptors on B cells express the Fab structural commitment of the B cells and their progeny AFC. Thus "sample" IgG molecules on the memory cell surface could serve as antigen receptors which allow the triggering of memory cells. The question of whether IgD is also present on these memory cells is under investigation. If they all bear IgD, then the question of which class of molecule serves as the receptor in triggering memory cells will have to await further study.

Strober* has recently also found IgG on IgG memory cells. His experiments also showed that these cells are small lym-

* Strober, S., *Maturation of B lymphocytes in rats. III. Two subpopulations of memory B cells in thoracic duct lymph differ by size, turnover rate and surface immunoglobulin, J. Immunol.*, in press.