# DEMONSTRATION OF HEAVY AND LIGHT CHAIN ANTIGENIC DETERMINANTS ON THE CELL-BOUND RECEPTOR FOR ANTIGEN

## SIMILARITIES BETWEEN MEMBRANE-ATTACHED AND HUMORAL ANTIBODIES PRODUCED BY THE SAME CELL*

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Initiation of immunological events following the exposure of immunocompetent cells to antigen is believed to include as an early essential step combination between preformed, cell-attached antibodies and immunogen. The exact location of this (these) inducing event(s) is unknown, but indirect information suggests this takes place at the outer surface of the relevant immunocyte. Cells of the lymphocytic series can be shown capable of selective binding of antigen to the outer cell membrane (1–3) and immunocompetent cells have enough surface-attached antibodies to allow selective retention when cells are allowed to filter through antigen-coated columns (4). It is well documented that certain small lymphocytes actively synthesize immunoglobulin-like molecules (5) which are on display on the cell surface (6, 7). That these latter molecules have antigen-binding specificity is suggested by the fact that adherence of antigen to lymphoid cells can be blocked by preincubating the cells with antibodies directed against serum immunoglobulins (8, 9). This latter finding demonstrates antigenic similarities between humoral antibodies and structures adjacent to the antigen-binding sites present on the surface of certain lymphoid cells. It does not provide any further information as to the possible identity of the two types of molecules.

According to a simple version, the membrane-bound, antigen-binding receptor merely represents and expresses the potential capacity of that cell to eventually produce that molecule in a more soluble form, i.e., as a humoral antibody. If so, the two types of antibodies would represent different distribution forms of the same molecule with only minor differences to account for differences in "solubility." We have attempted to further define the chemical build-up of membrane-attached antibodies by the combined use of fractionation of immune cells on antigen-coated column in the presence or absence of anti-immuno-

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globulin antibodies in the medium. If the surface receptor is immunoglobulin in nature it should be possible to block the antigen-binding capacity of that molecule by preincubating the cells before column passage with anti-immunoglobulin antisera. This turned out to be possible and information as to heavy and light chain antigenic specificities within receptor area could be gathered by the use of various anti-immunoglobulin class-specific antisera. The theoretical implications of these findings will be discussed.

## Materials and Methods

*Animals*—Adult, inbred mice of the strains A, BY, A.SW, CBA, DBA/2, and their respective $F_1$ hybrids were used as obtained from our own breeding colony. Within each experiment animals were of the same genotype and sex and approximately the same age. As the results obtained could be shown to be independent of strain of mice used, the genotype of the animals in the separate experiments will not be reported.

*Antisera*—A rabbit antiserum against "mouse serum immunoglobulins," hereafter referred to as a rabbit–anti-mouse poly Ig antiserum, was provided by Dr. B. Andersson through immunization of rabbits with *Salmonella adelaide* heat-killed bacteria coated with mouse–anti-*Salmonella adelaide* antibodies. This antiserum could be shown in gel diffusion to contain antibodies directed against both heavy- and light-chain determinants of all mouse immunoglobulin classes, and was obtained by pooling the antisera of seven different rabbits. Rabbit and goat antisera specific for gamma 1 or gamma 2a mouse immunoglobulin were kindly prepared and tested for specificity by Dr. F. Hymes, Immunoglobulin Reference Center, Melpar Inc., Falls Church, Va. Further tests as to the specificity of these antisera were carried out in this laboratory using the antisera as specific developers for hemolytic plaque-forming cells (PFC)[1] of that particular immunoglobulin class (10). Inhibition of plaque formation was attempted by addition of the purified gamma 1 or gamma 2a myeloma protein. The anti-gamma 1 antisera were all shown to be purely anti-gamma 1 by this test, whereas one of the anti-gamma 2a antisera could be shown to have some anti-gamma 1 reactive antibodies (a maximum of 30% inhibition of plaque formation by the addition of gamma 1 myeloma protein was obtained). Despite this finding, this latter antiserum functioned like the other anti-gamma 2a antisera in the column tests and the results obtained with it is included with the results gathered when using the other anti-gamma 2a sera. The individual antisera were used in concentrations related to their optimal capacity to function as developers for the class-specific PFC. A rabbit–anti-mouse kappa light chain antiserum was kindly provided by Dr. H. Wohl through the use of light chains from MOPC-21 (a gift from Dr. M. Potter) and the activity of this serum could be fully blocked in the present test by the addition of free kappa light chains to the antiserum before incubation with the cells. A rabbit–anti-mouse lymphocyte serum was produced by two intravenous injections of $4 \times 10^8$ lymph node cells 2 wk apart, bleeding the rabbits 1 wk after the last injection. No anti-mouse immunoglobulin antibodies were found in this serum as tested for by gel diffusion and capacity to function as developer for 7S hemolytic PFC. In cytotoxic test in vitro against mouse lymph node cells and rabbit complement, this antiserum could be shown to have a cytotoxic titer exceeding 1:1000     (11).

*Immunization*—Immunization against bovine serum albumin BSA, fraction V, Armour and Co., Eastbourne, England), ovalbumin OA, KEBO, Stockholm, Sweden), and 4-hydroxy-

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; EME, Eagle's medium in Earle's solution; NIP, 4-hydroxy-3-iodo-5-nitrophenyl acetic acid; OA, ovalbumin; PFC, plaque-forming cell(s).

3-iodo-5-nitrophenyl acetic acid (NIP) (12) coupled to BSA or OA to make $NIP_{10}$ BSA or $NIP_{11}$ OA conjugates was carried out using a 2 mg/ml concentration of either of these antigens in Freund's complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.). Injection of immunogen in adjuvant into the footpads of the hind legs was carried out. Boosting with antigen in transfer tests designed for the study of immunological memory was carried out using 20 μg of the antigen(s) per animal having the antigen dissolved in saline.

*Cells*—Immune cell suspensions for testing of high-rate antibody-forming cells in vitro after column passage or subsequently after transfer in an immunological memory test system were obtained from the popliteal lymph nodes and the spleens of mice immunized 1-6 months earlier. Single cell suspensions were prepared by squeezing the organs with a pair of forceps in ice-cold Eagle's medium in Earle's solution (EME) (SIBCO Grand Island Biological Co., Grand Island, N. Y.) followed by decantation and washing. All cell suspensions were kept at 4°C until tested in the different systems.

*Antibody Synthesis In Vitro*—Single, antibody-forming cells against BSA, OA, or NIP were enumerated in vitro using the hemolytic plaque assay (13), using rabbit or goat antimouse immunoglobulin antiserum to ensure detection of the relevant 7S plaque-forming cells (10). Target cells in the plaque assays were sheep erythrocytes that had been coated with either BSA or OA using bis-diazotized benzidine (10), or with NIP using NIP-azide at 1 mg/ml during labeling of the red cells (14). Using protein antigens, virtually no direct PFC of possible 19S character were found (15), but in the NIP system some 5% of the total number of PFC found were of direct type. This rather low number of direct PFC in the NIP system is due to the fact that cells were harvested for experiments at 1-6 months after primary immunization. The direct PFC figures in the NIP system were always subtracted from the 7S NIP PFC to give the reported 7S PFC numbers. It should be realized that direct PFC in the plaque system might be of either 19S or 7S type (14). In the blocking tests the same antisera that were used for preincubation of cells before fractionation on the columns were subsequently used to develop PFC in the hemolytic plaque tests.

*Tests for Immunological Memory*—The cells to be tested for immunological memory were administered intravenously into syngeneic mice that had been irradiated with 400 R the same day to ensure optimal antibody production of the inoculated cells (16). The number of cells inoculated varied between $10^6$–$15 \times 10^6$ as indicated in the various experiments, and antigen(s) in saline was administered together with the cells for boosting as indicated above. The animals were bled at either day 10 or 20 after transfer, and the sera assayed for antibody by the use of a modified ammonium sulphate precipitation assay (for details see reference 17) using isotope-labeled antigens. In the memory tests for specific gamma 1 and gamma 2a antibody synthesis, however, the spleens were harvested at day 10 after transfer, and analysis performed for gamma 1 and gamma 2a PFC using as developers for PFC the very same immunoglobulin class-specific antisera that had been used for blocking the cell-bound antibodies during columnar passage.

*Serological tests*—The concentration of humoral antibodies in the experimental animals directed against the various protein antigens was determined by the use of isotope-tagged antigens in an ammonium sulphate precipitation method (for details see references 16 and 17). The antibody titers are expressed in the tables as the amount of antigen in micrograms bound by 1 ml of antiserum at 50% antigen binding. In most experiments the antigen concentration during antibody titration was 0.1 μg/ml but in certain experiments, due to the low concentration of antibodies, titrations were carried out at lower concentrations of antigen. In each experiment all sera were titrated using the same antigen concentration used in the ammonium sulphate assay.

*Antigen-Coated Bead Columns*—Conditions for the preparation of antigen-coaten bead columns were as previously described (4, 17). Polymetaacrylic plastic particles with an average diameter of 250 μ (Degalan V26, Degussa Wolfgang AG, Hanau am Main, Germany) were

used as column materials, after having been labeled with the appropriate antigen, and were poured into glass tubes to make 1.5 × 100 cm glass columns. Free antigen was washed away and the columns were saturated with ice-cold EME and kept at 4°C during fractionation of cells, in order to reduce cellular metabolism and increase the efficiency of the columns to remove high-rate antibody-forming cells (4). The cells were filtered through the columns at a rate of approximately 2 ml/min. Retained cells were eluted from the beads by mechanical means (4) and control, passed, and retained cells were subsequently washed twice and were either analyzed for presence of PFC or used in a transfer system for the analysis of immunological memory.

*Blocking Experiments of Membrane Antibodies*—Immune cells were incubated at 4°C for 10 min with anti-lymphocyte, anti-immunoglobulin or normal goat or rabbit serum using, depending upon the strength of the antisera as tested in the indirect plaque assay, a final concentration ranging from 2 to 10% of serum. Subsequently, a sample of the cell suspension was applied to the top of an antigen-coated column and the cells were allowed to filter through in the presence of the serum used. In most experiments two or three parallel columns were run, coated with the same antigen but using different antisera for preincubation of samples of cells from the same cell population. After filtration the passed, retained, and control cells were washed twice and then either assayed immediately for PFC or tested for potential immune capacity in a transfer test as indicated above.

*Guide to Reading the Tables in Results*—The construction of the tables in the present article is based on the following observation: If immune cells against two antigens are transferred in an immunological memory test carrying out boosting with a mixture of the two antigens, within a given experiment the ratio of "anti-A" *vs* "anti-B" antibodies is very constant between the individual sera. The variation within the ratios of anti-A:anti-B in a given group is of similar order of magnitude as that observed when comparing the variation of anti-A or anti-B titers respectively (4, 17). On the other hand, the anti-A:anti-B ratio is not influenced by cell number transferred (4, 17) whereas it is well documented this influences the absolute anti-A and anti-B titers (16). Thus, in the present article major emphasis has been put on the impact on the anti-A:anti-B ratios when the cells have been through various experimental conditions. In several experiments different cell numbers have been transferred from the passed, retained, or control cells, thereby causing different absolute antibody titers or PFC numbers in the memory tests in the respective groups. The important question always asked, however, has been what is the impact of the treatment on the anti-A:anti-B ratios. Thus, in most tables (Tables I, II, III, V, and VII) the figures representing the relative change, if any, of the anti-A:anti-B antibody- or PFC-ratio are presented furthest to the right in each table. This ratio is expressed as a relative figure compared to the ratio obtained when using control cells.

## RESULTS

### Blocking of Specific Elimination of Immune Cells by Antigen-Coated Columns

*Effect of Rabbit–Anti-Mouse Poly Ig and Anti-Mouse Lymphocyte Antiserum.*—If the surface-attached receptors on immunocompetent cells (4) are immunoglobulin in nature it should be possible to block their antigen-binding capacity by anti-immunoglobulin antisera as has been previously found (8, 9). To link this blocking with a possible biological function of the receptor, this incubation of immune cells with anti-immunoglobulin antibodies was followed by a filtration of a sample of the cells through an antigen-coated column followed by

assessment of the immune capacity of the cells. A successful steric interference of the antigen-binding capacity of the membrane-bound antibody by anti-immunologobulin antibodies would, if causing no damage to the cell in an irreversible manner, manifest itself by allowing the relevant immune cells to pass through the antigen-coated column. Anti-BSA and anti-OA immune cells were pooled in vitro and samples of these cells were incubated at 4°C for 10 min with a 2% concentrated rabbit normal serum, rabbit–anti-mouse poly Ig, or rabbit–anti-mouse lymphocyte serum. Samples of the different suspensions were then allowed to filter through BSA-coated columns. The passed and the control cells were subsequently washed twice and then either analyzed immediately for high-rate antibody-synthesizing cells or tested in transfer system for immunological memory.

The results of experiments performed according to the above protocol are shown in Tables I and II. Incubation with anti-mouse poly Ig serum could be shown to significantly block the attachment of BSA PFC or immunological memory cells when cells were filtered through a BSA-coated column. This is indicated by the close to control values of anti-BSA:anti-OA reactivity in these cell suspensions. On the other hand, significant retention of BSA PFC and immunological memory cells was observed when the cells filtering through the BSA column had only been preincubated with rabbit normal serum. Most interesting, a similar "normal" reduction in BSA PFC was observed when the anti-mouse lymphocyte serum-treated cells sieved through the BSA-coated column. The efficiency of the serum was shown by its capacity to ablate the immunological memory in the transfer test as shown in Table II, thus amply verifying that antibodies from this antiserum had combined with surface structures on the cells, but not close enough to cause steric hindrance of the antigen-binding receptors. Summarizing, the data as presented in Tables I and II strongly suggest the presence of antigenic specificities common to serum immunoglobulins close to the antigen-binding areas on the surface of immune cells.

*Effect of Rabbit–Anti-Mouse Kappa Light Chain Serum.*—The predominating light chain group found in mouse serum antibody molecules is of kappa type (18). If the receptor molecules have a similar distribution pattern of light chains, most membrane antibodies would be of kappa-type as well, provided that light chains would participate in the build-up of these structures. In order to test for a possible presence of kappa light chain antigenic specificities within the close vicinity of the antigen-binding sites of membrane-attached antibodies, immune cells were preincubated with an anti-kappa light chain serum before passage through antigen-coated columns. Control groups included cells incubated with rabbit normal serum or rabbit–anti-mouse poly Ig serum. The immune capacity of the control and passed cells was assessed in the same way as in the preceding chapter.

The results shown in Table III demonstrate that preincubation with anti-kappa light chain as well as rabbit–anti-mouse poly Ig serum would allow the passage of immune cells through the relevant antigen-coated columns. No such blocking effect was observed when using a rabbit normal serum. Using the anti-kappa serum, a more than 50% reduction in the specific retention of immune

TABLE I

*Specific Elimination of High-Rate Antibody-Forming Cells by Passage Through Antigen-Coated Columns. Effect of Preincubating Cells with Rabbit–Anti-Mouse Lymphocyte, Rabbit–Anti-Mouse Poly-Ig, or Rabbit Normal Serum*

| Experiment | Column | Cells* | Serum‡ | BSA PFC§ | OA PFC§ | BSA PFC/ OA PFC | Reduction BSA PFC‖ |
|---|---|---|---|---|---|---|---|
| | | | | | | | (%) |
| 1 | BSA | C | Ig | 40 | 39 | 1.03 | 0 |
| | | C | ALS | 49 | 42 | 1.17 | 0 |
| | | C | NS | 48 | 23 | 2.09 | 0 |
| | | P | Ig | 14 | 9 | 1.56 | 0 (+9%) |
| | | P | ALS | 4 | 8 | 0.50 | 65 |
| | | P | NS | 6 | 19 | 0.32 | 78 |
| 2 | BSA | C | Ig | 12 | 30 | 0.40 | 0 |
| | | C | ALS | 18 | 54 | 0.33 | 0 |
| | | C | NS | 21 | 45 | 0.46 | 0 |
| | | P | Ig | 6 | 18 | 0.33 | 17 |
| | | P | ALS | 12 | 93 | 0.13 | 67 |
| | | P | NS | 18 | 141 | 0.13 | 67 |

* C, control cells; P, passed cells, Cell suspensions are a mixture of anti-BSA and anti-OA lymph node cells.

‡ Ig are cells preincubated with rabbit–anti-mouse poly-Ig serum prior to column passage; ALS are incubated with rabbit–anti-mouse lymphocyte serum; NS are incubated with rabbit normal serum before column filtration.

§ PFC values from duplicate plates for each suspension and antigen-coated RBC. Equal numbers of cells within each suspension tested for PFC:s against the two antigens, BSA or OA. Note, PFC values not corrected to PFC/$10^6$ cells.

‖ Relative reduction in anti-BSA PFC number as calculated from the BSA PFC/OA PFC ratios. Controls equal the mean of controls treated with Ig or NS equals 0% reduction.

cells was observed in experiment 1 (% reduction reduced from 99.6% to a mere 36% by pretreatment with anti-kappa); this showed a majority of cells in this test to have kappa light chain antigens in close proximity to the antigen-binding sites of their cell-bound antibodies.

*Effect of Rabbit–Anti-Mouse Gamma 1- or Gamma-2a Serum.*—In order to study in detail the possible existence of heavy chain antigenic specificities within the antigen-binding areas, with the eventual aim of demonstrating that the same heavy chain specificities within the receptor are present in the poten-

tial product of that cell, the humoral antibody, we now applied anti-heavy chain specific antisera to attempt to block the surface receptors. Immune cells were incubated with anti-gamma 1 or anti-gamma 2a serum and subsequently passed through antigen-coated columns. Passed, retained, mechanically eluted,

TABLE II

Specific Elimination of Immunological Memory Cells by Passage Through Antigen-Coated Columns. Effect of Preincubating Cells with Rabbit–Anti-Mouse Lymphocyte, Rabbit–Anti-Mouse Poly-Ig, or Rabbit Normal Serum

| Experiment | Column | Cells* | Serum‡ | Anti-BSA§ | Anti-OA§ | Anti-BSA/ Anti-OA | Reduction anti-BSA‖ |
|---|---|---|---|---|---|---|---|
| | | | | | | | (%) |
| 1 | BSA | C | Ig | −0.350 ±0.137 | −0.367 ±0.037 | 0.016 ±0.161 | 0 |
| | | C | ALS | all negative | all negative | — | — |
| | | C | NS | 0.439 ±0.284 | −0.063 ±0.116 | 0.501 ±0.217 | 0 |
| | | P | Ig | 0.050 ±0.213 | −0.377 ±0.201 | 0.427 ±0.146 | 0 (+47%) |
| | | P | ALS | all negative | all negative | — | — |
| | | P | NS | −0.391 ±0.122 | 0.109 ±0.149 | −0.499 ±0.199 | 83 |
| 2 | BSA | C | Ig | −0.164 ±0.063 | −0.401 ±0.172 | 0.237 ±0.135 | 0 |
| | | C | ALS | all negative | all negative | — | — |
| | | C | NS | 0.234 ±0.115 | −0.595 ±0.110 | 0.818 ±0.099 | 0 |
| | | P | Ig | 0.250 ±0.077 | −0.324 ±0.034 | 0.574 ±0.093 | 0 (+12%) |
| | | P | ALS | all negative | all negative | — | — |
| | | P | NS | −0.142 ±0.153 | −0.064 ±0.134 | −0.078 ±0.082 | 75 |

* C, control cells; P, passed cells. Cell suspensions are a mixture of anti-BSA and anti-OA lymph node cells.

‡ Ig are cells preincubated with rabbit–anti-mouse poly-Ig serum prior to column passage. ALS are incubated with rabbit–anti-mouse lymphocyte serum and NS are incubated with rabbit normal serum before column filtration.

§ Anti-BSA and anti-OA-antibody titers expressed in $\log_{10}$ numbers of mean ± standard error of the mean (for antibody titrations see Materials and Methods). Each group was comprised of five to six animals. Negative values represent antibody titers less than −1.000.

‖ Relative reduction in anti-BSA antibody titers as calculated from the anti-BSA/anti-OA ratios. Controls equal mean of controls treated with Ig or NS equals 0% reduction.

and control cells respectively were subsequently washed twice and assayed for presence of PFC or tested for memory capacity in a transfer system. In both test assays, the PFC of gamma 1 and gamma 2a class were developed using as facilitating antiserum the very same antiserum as had been used when prein-

cubating the cells before column passage. All cell suspensions were assayed fo ·
PFC of the two 7S antibody classes and the ratio of gamma 1:gamma 2a PFC
was calculated. A selective blocking of receptors belonging to one particular
immunoglobulin class by class-specific antiserum would be reflected in an en-
richment of these cells in the passed population and a corresponding relative
decrease among the retained cells. A positive outcome of such experiments
would strongly suggest identity as to heavy chain structures of the surface
receptor molecule and that of the potential product of that cell, the humoral
antibody.

TABLE III

Specific Elimination of Immunological Memory Cells by Passage Through Antigen-Coated
Columns. Effect of Preincubating Cells with Rabbit–Anti-Mouse Kappa Light
Chain Serum or Rabbit Normal Serum

| Ex-peri-ment | Column | Cells* | Anti-BSA‡ | Anti-NIP‡ | Anti-NIP/Anti-BSA‡ | Reduc-tion anti-BSA§ |
|---|---|---|---|---|---|---|
| | | | | | | (%) |
| 1 | BSA | CNS | 1.890 ± 0.104 | 2.598 ± 0.289 | 0.715 ± 0.221 | |
| | | PNS | 0.086 ± 0.171 | 3.030 ± 0.085 | 3.123 ± 0.195 | 99.6 |
| | | CLC | 1.640 ± 0.154 | 2.637 ± 0.275 | 0.997 ± 0.183 | |
| | | PLC | 1.490 ± 0.204 | 2.679 ± 0.257 | 1.189 ± 0.193 | 36 |
| | | | | | | |
| 2 | BSA | CNS | 1.132 ± 0.106 | 1.485 ± 0.073 | 0.352 ± 0.111 | |
| | | PNS | all negative | 2.587 ± 0.177 | 2.587 | 99.4 |
| | | CLC | 1.409 ± 0.086 | 1.857 ± 0.101 | 0.448 ± 0.104 | |
| | | PLC | 0.739 ± 0.276 | 2.165 ± 0.186 | 1.329 ± 0.097 | 87 |

* C, control cells; P, passed cells. NS are incubated with rabbit normal serum prior to
column passage. LC are incubated with anti-kappa light chain serum. Cells are a mixture of
immune BSA and NIP-OA spleen cells. Test for memory carried out as described in Mate-
rials and Methods.

‡ Anti-BSA and anti-NIP antibodies assessed as described in Materials and Methods.
Figures denote mean ± standard error of the mean. Five animals per group. Sera obtained at
day 10 after transfer. Figures in $log_{10}$ units. Negative values are antibody titers less than
−1.000.

§ Relative reduction in anti-BSA as calculated from the NIP/BSA ratios. Controls equal
0% reduction.

In the first set of experiments we analyzed any possible impact on the im-
mune capacity of cells incubated with the respective class-specific antisera, as
results obtained in other systems suggested that this might occur (19–22). The
results obtained in 10 assays of PFC ratios immediately after incubation and 5
experiments where the effect of incubation with antiserum was measured in
studies on immunological memory are shown in Table IV. In no group of anti-
serum-treated cells was there a significant change in the ratio of gamma 1 to
gamma 2a PFC as compared to the control figures arbitrarily put as 1 (con-

trols are cells not treated with anti-serum). Thus, no selective effect on high-rate antibody-forming cells nor on immunological memory cells could be demonstrated after incubation with anti-gamma 1 or anti-gamma 2a class-specific antisera. However, when measured in absolute numbers of PFC per unit cell number transferred in the immunological memory tests, a general reduction in immune capacity of cells preincubated with rabbit–anti-mouse immunoglobulin class-specific sera was observed ranging from 0 up to 25 % of

TABLE IV

*Lack of Effect of Incubation of High-Rate Antibody-Forming Cells or Immunological Memory Cells with Rabbit–Anti-Mouse Class-Specific Sera*

| High-rate antibody-forming cells (gamma 1/gamma 2a) | | | Immunological memory cells (gamma 1/gamma 2a) | | |
|---|---|---|---|---|---|
| Untreated* | Anti-gamma 1‡ | Anti-gamma 2a‡ | Untreated§ | Anti-gamma 1 | Anti-gamma 2a |
| 1.66 | 1.47 (0.89) | 1.19 (0.72) | 1.40 | 0.74 (0.53) | 2.04 (1.46) |
| 1.87 | 2.16 (1.16) | 1.65 (0.88) | 3.36 | 2.95 (0.88) | 2.15 (0.64) |
| 1.03 | 2.31 (2.24) | 1.30 (1.26) | 2.84 | 2.46 (0.87) | 1.39 (0.49) |
| 6.74 | 5.24 (0.78) | 10.47 (1.55) | 1.57 | 1.62 (1.03) | 2.74 (1.75) |
| 2.30 | 2.65 (1.15) | 1.47 (0.64) | 0.92 | 0.80 (0.87) | 0.68 (0.74) |
| 3.63 | 1.90 (0.52) | 1.96 (0.54) | | | |
| 2.90 | 2.80 (0.97) | 4.30 (1.48) | | | |
| 1.18 | 1.15 (0.97) | 1.22 (1.03) | | | |
| 2.34 | 2.58 (1.10) | 1.47 (0.63) | | | |
| 1.88 | 1.94 (1.03) | 1.55 (0.83) | | | |
| Total | 1.08 | 0.95 | | 0.84 | 1.01 |
| | ±0.17 | ±0.10 | | ±0.08 | ±0.07 |

* Gamma 1 PFC/gamma 2a PFC ratio in 10 separate experiments. Figures are mean of duplicate plates.

‡ As for untreated cells. Figures within brackets are the ratios compared to untreated equals 1. Bottom figures represent mean ± standard error of the mean.

§ Figures of the memory groups calculated as for the high-rate antibody-forming cells. Five separate memory tests. Each figure represents the mean of PFC ratios of the spleens of two mice analyzed separately.

the total reactivity. This reduction, however, is also seen quite frequently when using rabbit normal serum under similar conditions. It has not been possible to link this activity to presence of anti-immunoglobulin antibodies, but this effect is probably due to the presence in many rabbit sera of "natural" anti-mouse antibodies with unknown specificities.

Having failed to demonstrate any selective interfering with immune capacity of cells which have been in contact with anti-heavy chain-specific sera, we now studied the possible blocking effect of these antisera on the retention of immune cells by antigen-coated columns in the same way as previously described. In most experiments NIP-OA immune lymph node and spleen cells were incubated with the respective antisera followed by passage through $NIP_{10}$ BSA-

coated columns in order to allow selective retention pressure for the anti-NIP cells without trying to remove anti-OA cells. This was done in order to ensure that anti-carrier cells (anti-OA cells) would not be selected for or against in any cell fraction as $NIP_{11}$ OA was administered as boosting antigen in the memory tests where "helper" cells are needed for anti-hapten antibody formation to take place (23). Tests for NIP PFC were carried out as described in Materials and Methods, using as developer sera for gamma 1 and gamma 2a specific PFC the same antisera that had been applied during incubation before columnar passage.

TABLE V

*Effect of Rabbit–Anti-Mouse Immunoglobulin Class-Specific Sera on the Selective Elimination of High-Rate Antibody-Forming Cells Passing Through Antigen-Coated Columns*

| Cells* | NIP 1-PFC‡ | NIP 2a-PFC‡ | NIP 1- /NIP 2a-PFC§ |
|--------|-----------|-------------|---------------------|
| C | 93 | 56 | 1.66 (1.00) |
| C-1 | 53 | 42 | 1.26 (0.76) |
| P-1 | 53 | 11 | 4.82 (2.90) |
| R-1 | 17 | 27 | 0.63 (0.38) |
| C-2a | 43 | 36 | 1.19 (0.72) |
| P-2a | 34 | 33 | 1.03 (0.62) |
| R-2a | 43 | 10 | 4.30 (2.59) |

* $NIP_{11}$-OA immunized lymph node cells passed through a $NIP_{10}$-BSA coated column. C, control cells; P, passed cells. 1 and 2a are incubated with anti-gamma 1- or 2a-specific sera before column passage.

‡ Each figure represents the mean of three plates. PFC values are not corrected for the actual cell number plated.

§ The first figures equal the mean of the gamma 1 PFC/gamma 2a PFC ratio. Figures within brackets equal relative ratios as compared to C = 1.00.

In the first test group, the effect of incubation with class-specific antisera on the retention of high-rate antibody-forming cells by antigen-coated columns was analyzed. In all experiments two parallel columns coated with $NIP_{10}$ BSA were run using samples from the same original cell suspension incubated with anti-gamma 1 or gamma 2a serum. A total of 12 such experiments were performed and the results in absolute and relative PFC figures of one such experiment is shown in Table V. In this experiment incubation with anti-gamma 1 serum prior to column fractionation would cause a relative selective increase of gamma 1 anti-NIP PFC in the passed cells and a corresponding decrease in the retained cell population. Exactly opposite results were obtained when cells were incubated with anti-gamma 2a serum before column passage. The results suggest that high-rate antibody-forming cells express membrane-bound antibodies at 4°C with the same heavy chains as will be present in the humoral antibodies secreted by these cells at 37°C. In most experiments the selective

effect of preincubating immune cells with anti-heavy chain antisera before column filtration was weak but consistent. The results of 12 separate experiments are summarized in two $\chi$-square tables (Figs. 1 and 2). In these figures gamma 1:gamma 2a NIP PFC ratios in each passed or retained cell suspension has been compared to that of the control ratio put as 1. Data are grouped according to ratios larger or smaller than 1 and in each figure the effect of antisera against one heavy chain class have been grouped. Fig. 1 presents the mirror image of Fig. 2 and within each figure passed and retained cells show opposite distribution patterns. The $\chi^2$ value, calculated on a formula for testing association between blood groups (24, p. 76), is highly significant for both figures. Thus it would seem certain that high-rate antibody-forming cells, when put at 4°C, display antigen-binding receptor with the same heavy chain class as the humoral antibody produced by these cells.

|            | >1 | <1 |
|------------|----|----|
| P-gamma 1  | 11 | 1  |
| R-gamma 1  | 4  | 8  |

$\chi^2 = 8.71; 0.01 > p > 0.001$

Fig. 1

|             | >1 | <1 |
|-------------|----|----|
| P-gamma 2a  | 3  | 9  |
| R-gamma 2a  | 11 | 1  |

$\chi^2 = 10.92; p < 0.001$

Fig. 2

Fig. 1. Distribution pattern of gamma 1- /gamma 2a-PFC ratios as compared to control = 1. P, passed cells; R, retained cells. Gamma 1 is incubated with anti-gamma 1 sera before column passage. 12 experiments.

Fig. 2. As in Fig. 1 but here cells have been incubated with anti-gamma 2a serum before column filtration.

A control test was included again at this step to exclude the possibility that mere combination of anti-heavy chain-specific antibodies with a cell producing that immunoglobulin class would change the distribution pattern of these cells in such a way as to selectively enrich these cells in the passed population irrespective of antigen-binding specificity of their receptors. Cells immune against NIP, BSA, and OA were pooled and allowed to filter through BSA or OA columns in the presence of normal or anti-gamma 1 serum. The number of gamma 1 BSA or OA PFC were compared to those of gamma 1 or gamma 2a NIP PFC in the respective cell populations. A selective blocking of retention of gamma 1 OA or BSA PFC was observed when using anti-gamma 1 antiserum and OA or BSA columns; however, no change in the distribution pattern of gamma 1 versus gamma 2a NIP PFC was observed (see Table VI).

Using the above design but looking for heavy chain antigens within the receptors on memory cells we found (Table VII) a selective impact on cell passage through antigen-coated columns. Preincubation with an anti-gamma 1 antiserum prior to column filtration would selectively allow anti-NIP memory

TABLE VI

*The Effect of Preincubation of Immune Cells with Anti-Gamma 1 Serum Before Column Passage. A Comparison Between Cells Immune Against the Column Antigen and Cells Immune Against Another Antigen*

| Column* | Serum‡ | BSA 1/NIP 1§ | BSA 1/NIP 2a | OA 1/NIP 1 | OA 1/NIP 2a | NIP 1/NIP 2a |
|---|---|---|---|---|---|---|
| BSA | Anti-gamma 1 | 0.91 | 0.60 | n.d. | n.d. | 0.70 |
| BSA | Normal | 0.45 | 0.31 | n.d | n.d. | 0.75 |
| OA | Anti-gamma 1 | n.d. | n.d. | 0.59 | 0.63 | 1.04 |
| OA | Normal | n.d. | n.d. | 0.17 | 0.15 | 0.97 |

* Degalan columns coated with either BSA or OA.

‡ Cells preincubated prior to column passage with a 3% rabbit–anti-mouse gamma 1 or rabbit normal serum.

§ PFC ratios as analyzed in the passed-cell populations. Figures denote the relative ratios as compared to control cells equaling 1. Each figure is based on the results from duplicate plates. n.d., not done; 1, PFC of gamma 1 class; 2a, PFC of gamma 2a class.

TABLE VII

*Effect of Rabbit–Anti-Mouse Immunoglobulin Class-Specific Sera on the Specific Elimination of Immunological Memory Cells by Antigen-Coated Columns*

| Cells* | NIP 1-PFC‡ | NIP 2a-PFC‡ | NIP 1- /NIP 2a-PFC§ |
|---|---|---|---|
| C | 3108 | 896 | 3.36 (1.00) |
| C-1 | 1432 | 579 | 2.95 (0.88) |
| P-1 | 2772 | 188 | 14.63 (4.35) |
| R-1 | 327 | 238 | 1.32 (0.39) |
| C-2a | 1087 | 536 | 2.15 (0.64) |
| P-2a | 290 | 242 | 1.34 (0.40) |
| R-2a | 1219 | 202 | 5.41 (1.61) |

* Cells are immune NIP-BSA spleen cells; C are control cells; P are passed through a $NIP_{11}$-OA coated column. 1 and 2a represent in vitro incubation with rabbit–anti-mouse gamma 1 or 2a sera at 4% concentration for 10 min before column passage. R are retained cells, eluted by mechanical means. (For test of memory see Materials and Methods).

‡ Figures denote the sum of gamma 1- or gamma 2a-NIP PFC:s in two spleens in each group.

§ The first figure is the absolute mean gamma 1- /gamma 2a-NIP PFC of each group (mean of two individual ratios in each group). Figures within brackets represent relative ratios as compared to C = 1.00.

cells of gamma 1 type to "sneak through," causing a relative increase of the gamma 1:gamma 2a PFC ratios in the recipients of passed cells and a corresponding decrease in the retained group. Exactly the opposite distribution pattern of gamma 1:gamma 2a PFC was found when using an antigamma 2a serum. As for the high-rate antibody-forming cells, this effect by antisera was a weak but regular phenomenon. The results of all experiments performed are

summarized in two $\chi$-square tables (Figs. 3 and 4). Here, as in Figs. 1 and 2, the control mean gamma 1:gamma 2a PFC ratio was put as the index 1, and the ratios of the passed and retained recipient spleens have been ranked in groups smaller or larger than 1. As can be seen, Figs. 3 and 4 display very similar distribution patterns compared to Figs. 1 and 2, respectively, and again within each figure passed and retained cells incubated with the same antiserum display exactly opposite distribution patterns of gamma 1:gamma-2a PFC ratios. The $\chi$-square values indicate highly significant association factors between the ratios of the gamma 1:gamma 2a PFC in the antiserum-treated groups where a high ratio in the passed cells would come together with a low figure for the retained cells. The results obtained in the memory-cell system demonstrate two things: (a) memory cells have heavy chain-specific antigens

|  | >1 | <1 |
| --- | --- | --- |
| P-gamma 1 | 9 | 1 |
| R-gamma 1 | 0 | 10 |

$$\chi^2 = 16.36; p < 0.001$$

Fig. 3

|  | >1 | <1 |
| --- | --- | --- |
| P-gamma 2a | 2 | 19 |
| R-gamma 2a | 12 | 5 |

$$\chi^2 = 15.06; p < 0.001$$

Fig. 4

Fig. 3. Distribution pattern of potential gamma 1/gamma 2a memory ratios as compared to control = 1. P, passed cells; R, retained cells. Gamma 1 are incubated with anti-gamma 1 serum prior to column passage and memory test. Each unit represents the ratio in one spleen. Five experiments.

Fig. 4. As in Fig. 3 but here cells have been incubated with anti-gamma 2a serum before column filtration. Five different experiments.

in the close proximity of their antigen-binding sites on display on their outer surface and (b) the heavy chain of the membrane-bound antibody has antigenic specificities in common with the product of that cell in a future immune process, the humoral antibody.

## DISCUSSION

Knowledge of the chemical build up of surface receptors for antigens is essential for further understanding how an immunogen will trigger an immunocompetent cell of relevant specificity into "immunity" or "paralysis." Present information on this issue has been summarized in the introduction to this article and it strongly suggests that the surface receptor carries antigenic specificities in common with serum immunoglobulins (5–9, 19–22). Actual inhibition of immunocompetence of cells by treatment with anti-immunoglobulin serum has been achieved in varying experimental conditions (19–22) and in two of these (19–20) there is evidence that this takes place through a steric blockage of the uptake of antigen by the cell-bound antibody. None of the above results con-

tain any information as to the chemistry of the receptor in relation to the potential product of the very same cell (assuming the cell to belong to the bursa-associated system [25, 26]), the humoral antibody.

In the present article we approached this problem by the use of antigen-coated columns through which immune cells have been allowed to sieve in the presence of anti-immunoglobulin or other sera. Passed, retained, and control cells have subsequently been analyzed, either immediately or after a transfer for studying immunological memory, for the presence of high-rate antibody-forming cells by an in vitro test. In the first set of experiments it was shown that preincubation of immune cells with an antiserum directed against mouse immunoglobulins would allow the cells to pass through an antigen-coated column where they normally would be retained. In the same test another sample of the cells was incubated with an anti-mouse lymphocyte serum and this contact with antibodies, on the other hand, would not allow the cells to pass through the column. These experiments demonstrate that any antibody reacting with surface structures on immune cells will not block the antigen-binding receptors on the surface of these cells (4), but only certain antisera would do this, as in this case the anti-mouse immunoglobulin antibodies.

As a next step we used an antiserum specific for mouse kappa light chains as a blocker of the antigen-binding receptor in the column system. With this antiserum a highly significant blocking of the retention of relevant immuno-competent cells was observed, suggesting that in the majority of the immune cells there is a kappa light chain in the vicinity of the antigen-combining sites present on the surface of these cells. This is in agreement with the findings that in the mouse-serum immunoglobulins kappa is the dominating light chain type (18). The present findings would suggest a corresponding domination on the level of surface-bound antibodies.

The final test involved the use of anti-gamma 1 or gamma 2a sera as blockers of the retention of immune cells filtering through antigen-coated columns. Firstly, it was shown that mere incubation of immune cells with any of these antisera, followed by testing for immune capacity, would not detectably alter the reactivity of the cells. It was then found that preincubation of cells with an anti-gamma 1 antiserum would allow a selective sneaking through the antigen-coated columns by high-rate antibody-forming and immunological memory cells of the gamma 1 type, whereas gamma 2a cells with specificity towards the column antigen were retained in the ordinary fashion. Exactly opposite results were obtained when using an anti-gamma 2a antiserum. Control experiments incubating gamma 1 immune cells with an anti-gamma 1 antiserum, followed by filtration through a column coated with irrelevant antigen, demonstrated identical distribution patterns for gamma 1 and gamma 2a cells; this excluded the possibility that mere reaction with anti-immunoglobulin antibodies would change the distribution pattern of cells passing through any kind of column.

Taken together the present results strongly suggest the heavy chain of the surface receptor for antigen to be identical with the humoral antibody eventually produced by that cell. The data, although of more indirect nature, also indicate the presence of light chains within the surface receptor. Thus, the data suggest that the membrane-attached antibody is made up of heavy and light chain units in the same way as serum immunoglobulins. The present results are not informative as to whether the receptor molecule has the same number of antigen-binding sites as the humoral antibody of the same immunoglobulin class, but results of other workers indicate this to be the case (27, 28). It has previously been found that specificity and size of the antigen-combining site of the surface-attached antibody is similar if not identical to that of humoral antibodies directed against the same antigenic determinants (17, 19). Thus, in conclusion, membrane receptors for antigen and humoral antibodies seemingly represent two distribution forms of the same molecule with only possible minor differences to account for their different "solubilities."

The above statement is limited to the cell lines participating directly in humoral antibody formation. Using antigen-coated columns which allow immune cells to pass through, a selective retention of cells capable of humoral antibody synthesis against the column antigen is found, whereas cells immune against the same antigen but acting as "helper" cells for humoral anti-hapten antibody formation (23) could not be retained (29). Thus, using the present bead material in antigen-coated columns (4), we have no evidence that cells of thymic type (26) display receptors for antigen in sufficient quantities to allow specific retention when sieving through antigen-coated columns.[2] This finding, in conjunction with the results that the determination of immunoglobulin-class type resides within the cell potentially capable of humoral antibody formation (30), and the findings reported in the present article mean that a sizable part of the immunological memory in the 7S antibody system resides within the humoral antibody-forming cell lines.

## SUMMARY

High-rate antibody-forming cells and immunological memory cells can be selectively retained if filtered through a column coated with relevant antigen. This trapping can be blocked if the cells are incubated with an anti-immunoglobulin serum prior to column passage. A similar blocking is not observed when cells are treated with an anti-lymphocyte serum, thereby excluding the possibility that any antibodies combining with surface structures could cause this effect. By the use of antisera specific for heavy or light chain antigens, it was possible to locate such antigens in the antigen-binding receptor areas of immune cells. Criss-cross studies using antisera specific for gamma 1 or gamma

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[2] Wigzell, H. Characteristics of anti-carrier immune cells in hapten-carrier systems: differential separation of humoral antibody-forming cells and "helper" cells by passage through carrier-coated columns: To be published.

2a heavy chains showed that the membrane receptor has the same heavy chain as will be present in the eventual product of that cell, the humoral antibody.

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