INHIBITION OF THE IMMUNE RESPONSE BY 7S ANTIBODY
MECHANISM AND SITE OF ACTION*

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It is clear that passively administered 7S antibody can inhibit both the IgM and IgG responses and that the effect is specific with regard to antigen (1-3). Although there is general agreement on the effect of passively administered 7S antibody, there is no such agreement concerning the mechanism of this effect. Of the several possible mechanisms, the first and most obvious is that antibody simply combines with the majority of the antigenic determinants and neutralizes their effectiveness. With regard to the response to heterologous erythrocytes, it is clear that this mechanism cannot account entirely for the inhibition since severe suppression can be obtained at concentrations of antibody capable of combining with only a small proportion (<1%) of the available antigenic determinants (4-6). An alternative but closely related possibility is that the passively administered antibody causes the destruction or deviation of antigen before it contacts the cells in the antigen-sensitive unit. Once again, this mechanism does not easily account for the inhibition of humoral responses to heterologous erythrocytes, since antibody-mediated inhibition can occur under conditions where other antigen-dependent functions (e.g., antigen competition [7] and priming of thymus-derived (T) cells [8]) are not suppressed. Since direct effects on antigen appear unlikely to be the sole mechanism for antibody-mediated suppression, it has been suggested that antibody may act directly on one of the cells involved in initiation of humoral immune responses.

As shown by many investigations, the antigen-sensitive unit with respect to the response to heterologous erythrocytes is composed of bone marrow-derived (B) and T lymphocytes and nonlymphoid accessory (A) cells. Presumably, alteration of the function of any of these cells could result in suppression of the humoral response to heterologous erythrocytes. The in vivo experiments of Ryder and Schwartz (5) and the in vitro studies of Pierce (9) suggested that inhibition takes place at the level of the A

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Abbreviations used in this paper: A cell, nonlymphoid accessory cell; B cell, bone marrow-derived cell; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; GPS, guinea pig serum; HRBC, horse erythrocytes; N. A., nonadherent; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SRBC, sheep erythrocytes; T cell, thymus-derived cell.
The work of Feldmann and Diener (10, 11), using a slightly different in vitro system, suggested that inhibition takes place at the level of the B cell. In addition, Sinclair and Chan (12) have presented evidence that interaction of antigen, antibody, and lymphoid cells results in inactivation of the lymphoid cells.

The difficulty in interpreting the data referred to above is that the evidence implicating one or another cell type is indirect. Our approach to elucidating the cellular site of action of antibody has been to identify the cell type capable of reconstituting an inhibited spleen cell population. This should clearly identify the cell type which has been functionally inactivated by exposure to antibody. Using this approach, we have attempted to critically analyze a specific in vitro system in order to identify unequivocally the site of action of passively administered antibody. In this system, it is clear that antibody can act by combining with the A cell and inhibiting its normal function.

**Materials and Methods**

**Mice.**—The mice used were C57BL/6J x C3H/HeJ F1 hybrids (C3B6F1) (bred in our laboratories), C3H/HeDub (purchased from Flow Laboratories, Inc., Rockville, Md.) and DBA/2J (purchased from the Jackson Laboratory, Bar Harbor, Maine). All mice used were approximately 2 mo old.

**Antigens.**—Sheep erythrocytes (SRBC) and horse erythrocytes (HRBC) (obtained from Woodland Farms, Guelph, Ontario, Canada), washed three times in phosphate-buffered saline (PBS) and diluted to the appropriate concentration in PBS before intravenous injection, were used as antigens.

**7S Antibody Preparations.**—7S antibody was prepared from two sources: serum from hyperimmunized mice and ascitic fluid from hyperimmunized mice in which an ascites was induced by injection of Freund's complete adjuvant. The 7S fraction of the serum was isolated by Sephadex G-200 chromatography. The 7S fraction of the ascitic fluid was obtained by saturated ammonium sulfate precipitation followed by Sephadex G-200 chromatography. Normal 7S preparations were obtained in the same way, but from unimmunized mice.

**Assays.**—

*IgM-plaque-forming cells (PFC):* Splenic IgM-PFC were assayed using the technique of Jerne et al. (13) as modified by Kennedy et al. (14).

*Protein concentration:* Measurements of protein concentration were carried out according to the method of Lowry et al. (15).

*Hemolytic titer:* 7S antibody preparations were titrated using the microtiter system (Cooke Engineering Co., Alexandria, Va.). Twofold serial dilutions of the antibody were prepared. 0.025 ml of SRBC at a concentration of $4 \times 10^5$ SRBC/ml was added to 0.025 ml of diluted antibody. After 10 min incubation at 37°C, 0.025 ml of a mixture of 30% guinea pig serum (GPS) (as a source of complement) and 6% rabbit antismouse IgG serum (for augmentation of 7S-induced hemolysis) was added. This resulted in a final concentration of 10% GPS and 2% anti-IgG serum, concentrations which we had previously established as optimal. After 60 min incubation at 37°C, the titer was read as the reciprocal of the lowest antibody concentration providing apparently complete hemolysis. The serum-derived 7S antibody preparations had a facilitated hemolytic titer of $\sim 5,000$ at 1 mg/ml. The ascites-derived 7S antibody preparations had a facilitated hemolytic titer of $\sim 20,000$ at 1 mg/ml.

*Hemagglutination titer:* Hemagglutination titers were carried out as follows. Serial twofold dilutions (in PBS) of the antibody preparations were prepared to a volume of 0.1 ml in small (6 ml) plastic tubes. To each dilution was added 0.1 ml of SRBC at $1 \times 10^8$/ml.
tubes were incubated for 20 min at 37°C and then centrifuged at 150 g for 10 min. The agglutination titer was read as the reciprocal of the lowest concentration providing visible hemagglutination.

Culture System.—The method used was the double-chamber technique of Marbrook (16) as modified by Osoba (17). (The inner chamber volume was 1 ml and outer chamber volume was 9 ml.) The medium used was either 1066 with 20 μg/ml of L-asparagine added, or α medium, both supplemented with 10% fetal calf serum. Cultures were assayed for IgM-PFC on day 4. In our hands an optimum response was obtained at a cell density of 15-20 X 10⁶ spleen cells and 1 X 10⁶ erythrocytes/culture, and these concentrations were used in all experiments unless otherwise stated. All experimental points represent averages of the PFC measured in three to five cultures.

Irradiation.—Cells were irradiated using a 137Cs irradiator at a dose rate of 102 rad/min. Mice to be used as recipients for in vivo assays were exposed to 950 rad.

In Vivo Measurement of the Immune Response.—Cells to be assayed were injected into irradiated recipients along with antigen (10⁵ SRBC/recipient) and spleens were assayed for IgM-PFC on day 7. All groups consisted of 8-12 mice.

Cell Suspensions.—Spleen cell suspensions were obtained by pressing the tissue through a stainless steel screen as described previously (18). Peritoneal exudate cells were obtained by flushing the peritoneal cavity of exsanguinated normal mice with 5 ml of PBS.

Anti-θ Treatment of Cells.—Anti-θ antibody, prepared by injection of C3H thymus cells into AKR mice, was kindly provided by R. Gorczynski. This antibody has been shown to be specifically toxic for T cells, having no effect on B or A cells (19). Cells were incubated (in medium) at a concentration of 1 X 10⁶ cells/ml in the presence of 20% of the anti-θ preparation for 60 min at 37°C. The cells were then washed and resuspended (to 1 X 10⁷ cells/ml) in medium containing 20% GPS (as a source of complement). After 45 min incubation the cells were spun down and washed three times before culturing.

Sedimentation Analysis.—Velocity sedimentation cell separation, which separated cells primarily on the basis of size, was performed as described elsewhere (20). A glass sedimentation chamber 11.0 cm in diameter was used. A total of 30-40 X 10⁶ cells were loaded at a concentration of 10⁷ cells/ml in 0.2% bovine serum albumin (BSA) in PBS. 0.5-2% BSA in PBS-buffered step gradient was used (20). Cells were sedimented for 4 h at 4°C.

Conjugation of 7S with Fluorescein Isothiocyanate (FITC).—FITC conjugation was carried out as described by Holborrow and Johnson (21). The conjugation mixture consisted of 10 ml of the 7S preparation at a concentration of 1 mg protein/ml in a carbonate-bicarbonate buffer at pH 9, to which was added 2 mg FITC. This was stirred overnight at 4°C and dialyzed against PBS. Conjugation of mouse albumin was carried out similarly. The fluorescein to protein ratio, calculated as described by Holborrow and Johnson, was approximately 1:4:1 for both preparations.

Preparation of F(ab')₂ Fragments.—F(ab')₂ fragments of anti-SRBC 7S antibody were prepared by Dr. N. R. St. C. Sinclair as previously described (22).

Nonadherent (N.A.) Spleen Cells.—Nonadherent spleen cells were prepared using plastic tissue culture dishes as previously described (23). This population is deficient in A cells. Irradiation (1,000 rad) of nonadherent cells inactivates B and T cells, so that irradiated nonadherent spleen cells are deficient in all three classes of immunocompetent cells (A, B, and T cells).

RESULTS

Effect of 7S Antibody Treatment on the Immunocompetence of Spleen Cells.—The effect of 7S antibody on the immunocompetence of spleen cells was tested by incubating the cells with various concentrations of antibody, in the absence
of antigen, before culture in Marbrook chambers. The experimental procedure is illustrated in Fig. 1. Spleen cells were incubated for 1 h in the presence of various concentrations of anti-SRBC 7S antibody (control cells were incubated in the absence of antibody). The cells were then washed thoroughly to remove free 7S, and were cultured in Marbrook chambers in the presence of SRBC. After 4 days of incubation, all cultures were assayed for the presence of IgM-PFC to SRBC. The pooled results of six experiments are shown in Fig. 2, with the results plotted as PFC (percent of control) vs. concentration of 7S antibody present during treatment of spleen cells. Treatment with 7S antibody reduced the immunocompetence of the spleen cells, and the effect was dose dependent; the slope of the curve shown in this figure is $-0.47 \pm 0.07$.

It is of interest that the addition of either 7S antibody or antibody-coated SRBC to cultures causes a dose-dependent inhibition with similar kinetics (6). The inhibition curve for addition of 7S antibody has a slope of $-0.51 \pm 0.07$. The inhibition curve for addition of SRBC coated with different amounts of antibody has a slope of $-0.51 \pm 0.06$. In view of the similar kinetics of inactivation for all three cases, it is possible that they act through the same mechanism. Since pretreatment of cells with antibody is most amenable to the investigation of the mechanism, this procedure was examined more fully.

![Diagram of experimental procedure](image)
Fig. 2. Immunocompetence of 7S-treated spleen cells. Spleen cells were treated with graded concentrations of anti-SRBC 7S antibody, as illustrated in Fig. 1, before being cultured (20 X 10^6 cells/culture) with 10^6 SRBC. The 7S antibody concentration represents the titer present during spleen cell treatment. The points represent the geometric mean (GM) of PFC per culture ± standard errors (SE) expressed as percent of untreated control. The pooled results of six experiments are represented by different symbols. The average control value for the six experiments was 950 PFC/culture. The slope of the best-fitted straight line (least-squares method) is \(-0.47 ± 0.07\) (standard deviation). Cells: ○, □, ■, △ = C3B6F1; △ = DBA. 7S antibody: ○, □, ■, △ = C3B6F1; △ = C3H.

Although the most interesting interpretation of the data in Fig. 2 is that the antibody directly affects one of the cells in the antigen-sensitive unit, there are several alternative explanations for the observed suppression. They are discussed below.

**Carry-over of free 7S antibody:** The simplest explanation for the suppression observed in Fig. 2 is that it results from carry-over of free 7S antibody, since it is known that addition of 7S antibody to cultures results in an inhibition of the response (9, 10, 24). To test for this possibility, the four supernatants obtained from the cell washings after 7S treatment (see Fig. 1) were added to cultures containing normal spleen cells and antigen. Each supernatant was added, to a final concentration of 20%, to cultures containing 2 X 10^7 normal spleen cells and 1 X 10^6 SRBC. Since, after the final wash, the cells were always diluted at least 20-fold by volume before culturing, the amount of 7S added to the cultures in the final supernatant was always greater than that which would be carried over under experimental conditions. The results of these experiments are shown in Table I. In all cases, there was no detectable inhibitory effect by the supernatant from the final wash. This demonstrates that there is insignificant carry-over of free 7S antibody to the cultures under our standard experimental conditions. The possibility that antibody is released by treated cells is considered in the next section.

**Deactivation of antigen or release of free 7S antibody into cultures:** Since suppression is not the result of transfer of free 7S antibody, it follows that treatment of spleen cells with 7S antibody results in an alteration of one or more
TABLE I

| Supernatant added* to cultures (20%) | PFC (% of control¶) | Exp. 1 | Exp. 2 | Exp. 3 |
|-------------------------------------|---------------------|--------|--------|--------|
| -- (control)                        | 100 (76-132)§       | 100 (81-123)∥ | 100 (82-122)¶ |
| 1 (from treatment mixture)          | <1                  | <1     | Not done |
| 2 (from 1st wash)                   | 15 (9-19)           | 16 (13-19) | Not done |
| 3 (from 2nd wash)                   | 77 (56-107)         | 64 (47-86) | Not done |
| 4 (from 3rd wash)                   | 108 (77-150)        | 101 (94-107) | 108 (103-114) |

* Spleen cells were treated with anti-SRBC 7S antibody as illustrated in Fig. 1. Spleen cell treatment was carried out at a 7S antibody titer of 2,000 for exps. 1 and 2, and 5,000 for exp. 3. (The response of the treated spleen cells was 8-12% of controls for these three experiments.) After treatment, the spleen cells were washed as described in Fig. 1, and the supernatants were collected. The supernatants were then added, to a final concentration of 20%, to cultures containing 20 × 10^6 normal spleen cells and 10^6 SRBC. Cells, C3B6F1; 7S antibody, C3B6F1.

† PFC given as percent of control ± standard error.

§ Control value (100%), 400 PFC/culture.
∥ Control value, 800 PFC/culture.
¶ Control value, 1,000 PFC/culture.

classes of cells in the spleen. This may be the result of an alteration of one of the three cell types (A, B, or T) involved in the initiation of the response in such a way that the cell is rendered immunologically incompetent. Alternatively, the effect may be mediated by a different class of cells with which the 7S antibody has become associated. This latter effect could occur if (a) the presence of this 7S-associated cell in the cultures would result in destruction of the antigen or deviation of the antigen from the immunocompetent cell compartment (see, for example, reference in footnote 2) or, (b) if 7S antibody could be released from this cell into the culture medium where it could act by a different mechanism.

In order to test for these possibilities, mixtures of treated and untreated spleen cell populations were cultured. If either of the two mechanisms suggested above were operating, the response of normal cells should be greatly inhibited by the presence of the 7S treated cells in the same culture. If, on the other hand, 7S treatment results in one of the immunocompetent cell types being rendered incompetent, the untreated cells should respond normally.

Two types of mixing experiments were done. Since cell density is critical for the culture system, in all experiments the total cell concentration was kept constant at 2 × 10^7 spleen cells/culture. In the first design, the response produced by a mixture of 1 × 10^7 7S-treated cells plus 1 × 10^7 untreated cells was

2 MacDonald, H. R., R. A. Phillips, and R. G. Miller. Antibody-dependent cell-mediated cytotoxicity: evidence favouring non-lymphocytic effector cells. Manuscript in preparation.
compared with that produced by $2 \times 10^7$ untreated cells (Table II, exps. 1 and 2). The results show that the response obtained from $1 \times 10^7$ untreated cells in the presence of $1 \times 10^7$ 7S-treated cells is approximately one-half that produced by $2 \times 10^7$ untreated cells. In the second design, the response produced by a mixture of $1 \times 10^7$ 7S-treated cells plus $1 \times 10^7$ untreated cells was compared with that produced by $1 \times 10^7$ untreated cells (Table II, exp. 3). For the latter group, the total cell concentration was made up to $2 \times 10^7$ cells/culture by the addition of $1 \times 10^7$ irradiated nonadherent (N.A.) spleen cells (see Materials and Methods). These cells were used to adjust the cell density since they do not contain any functional cells of the three types involved in the immune response (23). In this experiment, $1 \times 10^7$ untreated spleen cells gave the same response in the presence or absence of $1 \times 10^7$ 7S-treated cells.

In both types of mixing experiments, there is clearly no severe inhibition of the untreated cell population by the 7S-treated cell population. Therefore, we can conclude that the inhibition is not the result of either antigen deviation or release of free 7S antibody from treated cells, but the inhibition is probably the result of an alteration of one of the three classes of cells (A, B, or T) involved in the initiation of the immune response.

**Nonspecific cytotoxicity and specificity:** The previous data suggest that, under the conditions tested, 7S antibody acts by rendering specifically incompetent one or more of the cells required for initiation of antibody produc-

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**TABLE II**

*Spleen Cell Treatment: Mixing Experiments*

| Exp. | Untreated spleen cells | 7S-treated spleen cells | Irradiated N.A. spleen cells | PFC (% of control) |
|------|------------------------|------------------------|----------------------------|-------------------|
| 1*   | $2 \times 10^7$        | 0                      | 0                          | 100 (99-101)      |
|      | $1 \times 10^7$        | $1 \times 10^7$        | 0                          | 46 (41-52)        |
| 2†   | $2 \times 10^7$        | 0                      | 0                          | 100 (93-107)      |
|      | $1 \times 10^7$        | $1 \times 10^7$        | 0                          | 51 (47-56)        |
| 3‡   | $1 \times 10^7$        | 0                      | $1 \times 10^7$            | 100 (93-107)      |
|      | $1 \times 10^7$        | $1 \times 10^7$        | 0                          | 98 (92-103)       |

* All cultures contained $10^6$ SRBC. Control value, 1,600 PFC/culture. 7S treatment carried out at titer of 5,000. Cells, C3B6F1; 7S antibody, C3B6F1.
† Control value, 1,700 PFC/culture. 7S treatment carried out at titer of 5,000. Cells C3B6F1; 7S antibody, C3B6F1.
‡ Control value, 700 PFC/culture. 7S treatment carried out at titer of 2,000. Cells, C3B6F1; 7S antibody, C3B6F1.
tion. Alternatively, the effect could be the result of nonspecific cytotoxicity by a component of the 7S preparation. To test for such cytotoxicity, spleen cells were treated with anti-SRBC 7S or normal 7S (isolated from the serum or ascitic fluid of normal mice) as illustrated in Fig. 1. After being washed, the cells were cultured in the presence of SRBC and their ability to respond was compared with that of controls. The results of these experiments are shown in Table III. Treatment with normal 7S had little, if any, effect on the ability of

| Exp. | Spleen cell treatment | 7S antibody | Source* | Concentration | PFC (% of control) |
|------|----------------------|--------------|---------|---------------|-------------------|
| 1§   | — (Control)          | —            | —       | —             | 100 (92-109)      |
|      | Anti-SRBC 7S         | Serum        | 0.75    | 3,750         | 30 (27-33)        |
|      | Normal 7S            | Serum        | 0.75    | —             | 98 (87-100)       |
| 2‖   | — (Control)          | —            | —       | —             | 100 (89-112)      |
|      | Anti-SRBC 7S         | Ascites      | 0.075   | 1,500         | 22 (19-25)        |
|      | Normal 7S            | Ascites      | 0.15    | —             | 93 (76-113)       |
| 3¶   | — (Control)          | —            | —       | —             | 100 (87-115)      |
|      | Anti-SRBC 7S         | Ascites      | 0.04    | 800           | 25 (21-30)        |
|      | Normal 7S            | Ascites      | 0.2     | —             | 92 (76-113)       |

* 7S antibody was derived either from serum of C3B6F₁ mice or from ascites of C3H mice, as described in Materials and Methods. Anti-SRBC 7S antibody was prepared from serum or ascites of mice hyperimmunized against SRBC; normal 7S was prepared in an identical manner from serum or ascites of unimmunized mice. Serum-derived anti-SRBC 7S antibody has a titer (anti-SRBC) of 5,000 at 1 mg/ml; ascites-derived anti-SRBC 7S antibody has a titer of 20,000 at 1 mg/ml.

† All titers, here and elsewhere, are hemolytic titers unless otherwise stated.

§ All cultures contained 20 X 10⁶ spleen cells and 10⁶ SRBC. Control value (100%), 2,300 PFC/culture. Cells, C3B6F₁.

‖ Control value, 1,200 PFC/culture. Cells, C3B6F₁.

¶ Control value, 800 PFC/culture. Cells, DBA.

the cells to mount an immune response to SRBC, while treatment with the same concentration, or lower, of anti-SRBC 7S reduced the immunocompetence of these cells. Thus, the suppressive effect is not the result of nonspecific cytotoxicity in the isolated component of mouse serum or ascitic fluid.

The specificity of the effect with regard to different antigens was then tested. Spleen cells were treated with equal concentrations of either anti-SRBC 7S or anti-HRBC 7S, washed, and cultured in the presence of one or the other of the antigens. The results are shown in Table IV. Treatment with anti-SRBC 7S reduced the ability of the cells to respond to SRBC but not to HRBC.
### MECHANISM OF 7S INHIBITION

#### TABLE IV

| Exp. | Spleen cell treatment | 7S concentration | Antigen | PFC* (% of control) |
|------|-----------------------|-------------------|---------|---------------------|
| 1§   | —                     | —                 | SRBC    | 100 (94–106)        |
|      | Anti-SRBC 7S          | 0.5               | SRBC    | 24 (20–28)          |
|      | Anti-SRBC 7S          | 0.5               | HRBC    | 100 (90–111)        |
|      | —                     | —                 | SRBC    | 108 (98–119)        |
| 2|| | Anti-HRBC 7S          | 0.5               | SRBC    | 100 (86–117)        |
|      | —                     | —                 | HRBC    | 81 (72–92)          |
|      | Anti-HRBC 7S          | 0.5               | HRBC    | 100 (76–132)        |
|      | —                     | —                 | HRBC    | 19 (13–29)          |

* PFC are anti-SRBC PFC when SRBC is used as antigen, and anti-HRBC PFC when HRBC is used as antigen.

† 7S antibody concentrations are expressed as protein concentrations rather than hemolytic titers, since we found that the hemolytic efficiency of anti-HRBC 7S antibody is not the same as anti-SRBC 7S antibody. The 7S antibody is serum derived; the titer of the anti-SRBC 7S antibody is 2,500 at 0.5 mg/ml.

§ Control values: 2,100 PFC/culture vs. SRBC; 200 PFC/culture vs. HRBC. Cells, C3B6F1; 7S antibody, C3B6FI.

‖ Control values: 1,000 PFC/culture vs. SRBC; 200 PFC/culture vs. HRBC. Cells, C3B6F1; 7S antibody, C3B6FI.

Similarly, treatment of cells with anti-HRBC 7S altered their response to SRBC only slightly, while severely inhibiting the response to HRBC. The effect, therefore, is antigen specific.

**Cellular Site of Action of 7S Antibody-Mediated Inhibition.**—Since treatment of spleen cells with specific 7S antibody results in a functional depletion of one of the immunocompetent cells, identification of the depleted cell type provides a method for elucidating the cellular site of action of 7S inhibition. Results from other groups (5, 25) have demonstrated that 7S antibody-treated spleen cells respond normally to erythrocyte antigens when assayed in irradiated recipients. However, the in vivo assay system requires only functional B and T cells, since radiation-resistant A cells are supplied by the recipients (23). The in vitro system, on the other hand, requires immunocompetent cells of all three types. This indirect reasoning infers that the cell being affected by our treatment is the radiation-resistant A cell.

In order to test this hypothesis, three different assay systems were used:

(a) The standard in vitro system, which requires competent A, B, and T cells.

(b) The standard in vivo system, in which spleen cells and SRBC are injected into recipients 2 h after irradiation and spleens are assayed for IgM-PFC 7 days later. This system requires immunocompetent B and T cells in the inoculum, A cells being supplied by the recipient.
The in vivo system of Gorczynski et al. (23) in which mice are irradiated 72 h before injection of spleen cells and SRBC. These recipients are deficient in A cells as well as B and T cells, and thus provide an in vivo assay system in which immunocompetent cells of all three types are required.

The immunocompetence of control and anti-SRBC 7S-treated spleen cells (treatment as in Fig. 1) was tested in all three systems. The results are shown in Table V. A reduction in the immunocompetence of spleen cells as a result of

| Exp. | Spleen cell treatment | In vitro PFC (% of control) | 2 h-irradiated recipients§ | 72 h-irradiated recipients§ |
|------|-----------------------|-----------------------------|-----------------------------|-----------------------------|
| 1    | (Control) 7S§         | 100 (87-115)                | 100 (90-111)                | 100 (81-123)                |
|      | 7S§                   | 15 (11-19)                  | 101 (88-117)                | 25 (20-32)                  |
| 2    | (Control) 7S§         | Not done                    | 100 (78-128)                | 100 (87-115)                |
|      | 7S§                   | Not done                    | 65 (45-94)                  | 11 (7-17)                   |
| 3    | (Control) 7S§         | 100 (74-136)                | 100 (86-116)                | Not done                    |
|      | 7S§                   | 24 (20-28)                  | 160 (131-195)               | Not done                    |

Average % of control for 7S-treated cells: 20 108 18

* For the in vitro assay, 15 × 10⁶ spleen cells were cultured with 1 × 10⁶ SRBC. PFC were assayed on day 4. For the in vivo assays, 15 × 10⁶ spleen cells were injected with 1 × 10⁹ SRBC. PFC were assayed on day 7.

§ Control values:

|             | Exp. 1 | Exp. 2 | Exp. J |
|-------------|--------|--------|--------|
| In vitro (PFC/culture) | 700    | Not done | 400    |
| 2 h recipients (PFC/spleen) | 500    | 600    | 400    |
| 72 h recipients (PFC/spleen) | 900    | 600    | Not done |

|| 7S treatment of spleen cells as illustrated in Fig. 1. Treatment was carried out with anti-SRBC 7S antibody at a titer of 3,000. (7S antibody, C3H; cells, C3B6F₁). 7S treatment was observed in the in vitro system and in the 72 h-irradiated recipients, but not in the 2 h-irradiated recipients. It is clear that the defect caused by 7S treatment is detected only in systems which require functional A cells.

Reconstitution.—The results described above suggest that 7S treatment of spleen cells results in a functional depletion of A cells. If this is the case, it should be possible to reconstitute a 7S-treated spleen cell population by addition of A cells. It has been shown that peritoneal exudate cells provide a rich source of A cells (26).
The reconstituting ability of A cells was tested by culturing $15 \times 10^6$ 7S-treated and control spleen cells in the presence or absence of $5 \times 10^5$ peritoneal cells. The results are shown in Table VI. Treatment of spleen cells with 7S antibody resulted in 93% inhibition of the response (groups 1 and 2). Group 3 demonstrates that the 7S-treated spleen cells can be completely reconstituted by the addition of peritoneal cells.

The increase in the response provided by the peritoneal cells may be due to the replenishment of the cell type which has been altered by 7S treatment; alternatively, the effect may be the result of an unrelated synergism between the spleen cells and peritoneal cells. Two pieces of evidence make the latter interpretation unlikely. First, there is clearly no synergism between peritoneal cells and untreated spleen cells (Table VI, group 4). Secondly, the peritoneal

| Group | Spleen cells | Peritoneal cells | PFC (% of control) |
|-------|--------------|------------------|--------------------|
|       | Treatment    | Cells/culture    | Treatment          | Cells/culture    |                |
| 1     | ---          | $15 \times 10^6$ | ---                | 0                | 100 (82-122)* |
| 2     | 7S‡          | $15 \times 10^6$ | ---                | 0                | 7 (6-8)        |
| 3     | 7S           | $15 \times 10^6$ | ---                | $5 \times 10^5$  | 120 (110-130) |
| 4     | ---          | $15 \times 10^6$ | ---                | $5 \times 10^5$  | 90 (83-97)     |
| 5     | 7S           | $15 \times 10^6$ | 7S§                | $5 \times 10^5$  | 33 (27-40)     |
| 6     | ---          | $15 \times 10^6$ | 7S                 | $5 \times 10^5$  | 101 (87-119)   |

* All cultures contained $10^6$ SRBC. Control value, 550 PFC/culture.
‡ Spleen cells (C3B6F1) were treated with anti-SRBC 7S antibody in the standard way (see Fig. 1) at a titer of 5,000. (7S antibody, C3B6F1.)
§ 7S treatment of peritoneal cells was carried out in exactly the same manner as 7S treatment of spleen cells.

The cells are themselves sensitive to 7S treatment: their ability to reconstitute was severely reduced by 7S antibody treatment (group 5). Since the treated peritoneal cells did not inhibit the response provided by normal spleen cells (group 6), the treated cells could not have transferred significant amounts of free 7S antibody. These data indicate that reconstitution is the result of the addition to the spleen cells of a particular class of cells and that this class of cells can be functionally depleted by 7S treatment.

Characterization of the Reconstituting Cell.—The results presented above suggest that the cell type which provides reconstitution is the same as that which is altered by 7S antibody treatment of spleen cells. This then provides an independent method of characterizing the affected class of cells.

Of the three cell classes involved in the initiation of an immune response to heterologous erythrocytes, two are radiation sensitive (B and T) and one is radiation resistant (A). In order to characterize the reconstituting cell in this
regard, peritoneal cells were irradiated (1,000 rad) before being added to 7S-treated spleen cells. The results, shown in Table VII, demonstrate that the ability to reconstitute is radiation resistant, indicating that the reconstituting cell, and thus by inference the cell in the splenic population which is altered by 7S treatment, is the radiation-resistant accessory cell. The remaining groups in this table are further controls demonstrating that 5 × 10^6 peritoneal cells, whether irradiated or not, do not themselves produce any PFC in cultures made up to optimal cell density with irradiated spleen cells.

The quantification of the ability of irradiated peritoneal cells to reconstitute 7S-treated spleen is shown in Fig. 3. Different concentrations of irradiated peritoneal cells were added to control and 7S-treated spleen cells, and the mixtures were cultured as usual. While the peritoneal cells did not significantly enhance the untreated spleen cell response at any of the given concentrations, significant reconstitution of the 7S-treated spleen cells was obtained at a concentration of 5 × 10^6 peritoneal cells.

There is evidence that some T cell functions are radiation resistant (see reference 27 for discussion). To demonstrate that reconstitution of 7S-treated spleen cells is not the result of a radiation-resistant T cell function, the sensitivity of the reconstituting cell to anti-0 antibody was tested. In these experiments, peritoneal cells were treated with anti-0 antibody as described in Materials and Methods before being added to 7S-treated spleen cells. The results, along with the appropriate controls, are shown in Table VIII. It is clear that the reconstituting ability of peritoneal cells is resistant to treatment with anti-0 antibody.

**Sedimentation Analysis of the Reconstituting Cell.**—The three immunocompetent cell classes present in the spleen have been characterized with respect to

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**TABLE VII**

| Treatment | Cells/culture | Peritoneal cells | PFC (% of control) |
|-----------|---------------|-----------------|--------------------|
|           |               | Treatment | Cells/culture | Exp. 1* | Exp. 2† |
| —         | 15 × 10^6     | —        | 0             | 100 (88-114) | 100 (85-118) |
| 7S§       | 15 × 10^6     | —        | 0             | 22 (17-28)    | 3 (1-4)     |
| 7S        | 15 × 10^6     | 1,000 rad | 5 × 10^5     | 80 (73-88)    | 14 (109-140)|
| —         | 15 × 10^6     | 1,000 rad | 5 × 10^5     | 78 (75-82)    | 102 (96-107)|
| 1,000 rad | 15 × 10^6     | —        | 0             | Not done      | <0.1        |
| 1,000 rad | 15 × 10^6     | —        | 5 × 10^5     | Not done      | <0.1        |
| 1,000 rad | 15 × 10^6     | 1,000 rad | 5 × 10^5     | Not done      | <0.1        |

* All cultures contained 10^6 SRBC. Control value, 1,000 PFC/culture. (Cells, C3B6F1.)† Control value, 2,300 PFC/culture. (Cells, C3B6F1.)§ Spleen cells treated with anti-SRBC 7S antibody at a titer of 5,000. (7S antibody: exp. 1, C3B6F1; exp. 2, C3H.)
Fig. 3. Quantification of reconstituting ability of irradiated peritoneal cells. $1.5 \times 10^8$ untreated (●) or 7S-treated (○) spleen cells were cultured with graded doses of irradiated (1,000 R) peritoneal cells (and SRBC). Points are GM ± SE as percent of control. The control value (untreated spleen cells alone) was 850 PFC/culture. Cells, C3B6F1; 7S antibody, C3H. (7S treatment of cells was carried out at a titer of 3000.)

The ability of spleen cells to reconstitute after 3 days of culture followed by irradiation (1,000 rad) is shown in Table IX. These cells provide significant reconstitution and the reconstituting ability is sensitive to 7S treatment. These cells were, therefore, used as a spleen-derived source of cells for sedimentation analysis of the reconstituting cell population.

Sedimentation was carried out as described in Materials and Methods. After sedimentation, fractions were combined into four pools, irradiated (1,000 rad),
### TABLE VIII

*Anti-O Sensitivity of Reconstituting Cell*

| Spleen cells | Peritoneal cells | PFC (% of control) |
|--------------|------------------|-------------------|
| Treatment    | Treatment        |                   |
|              | Cells/culture    |                   |
| —            | —                | 100 (90–111)      |
| 7S§          | 15 × 10^6        | 5 (4–6)           |
| 7S           | 15 × 10^6        | 72 (63–82)        |
| —            | 15 × 10^6        | 79 (66–94)        |
| —            | 15 × 10^6        | Not done          |
| Anti-θ        | 15 × 10^6        | 85 (74–98)        |
| C’           | 15 × 10^6        | 96 (81–113)       |
| Anti-θ + C’  | 15 × 10^6        | 2 (1–3)           |

* All cultures contained 10^6 SRBC. Control value, 400 PFC/culture. (Cells, C3B6F1.)

‡ Control value, 500 PFC/culture. (Cells, C3B6F1.)

§ 7S treatment of spleen cells was carried out, in the usual manner, with anti-SRBC 7S antibody at a titer of 1,000. (7S antibody, C3B6F1.)

¶ Peritoneal cells were treated with C’ alone (as control) or anti-0 antibody followed by C’ as described in Materials and Methods.

‖ In order to test the effectiveness of the anti-θ treatment, spleen cells were treated with anti-θ antibody alone, GPS (C’) alone, or anti-θ antibody followed by C’ as described in Materials and Methods.

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* and added back to 7S-treated spleen cells to a final concentration of 15 × 10^6 7S-treated spleen cells plus 1.5 × 10^6 irradiated, fractionated cells/culture. The results are shown in Fig. 4. The base line is provided by 15 × 10^6 7S-treated cells alone, and the control by 15 × 10^6 untreated spleen cells. As can be seen, substantial reconstitution was provided only by cells with a sedimentation velocity of >5 mm/h. This observation provides two pieces of information. First, the reconstituting cell is a distinct class of cells, separate from the major cell peak; therefore, reconstitution is unlikely to be the result of a nonspecific “feeder” effect. Secondly, the sedimentation velocity of the reconstituting cell is similar to that of A cells (4–6 mm/h) and not to B and T lymphocytes, which have sedimentation velocities in the range of 3–4 mm/h.

To verify that the sedimentation velocity of the reconstituting cell in normal spleen is the same as that in 3-day-cultured spleen, sedimentation analysis of the reconstituting cell in normal spleen was carried out. After sedimentation of normal spleen cells, fractions were combined into three pools, irradiated, and added back to 7S-treated spleen cells to a final concentration of 15 × 10^6 7S-treated spleen cells plus 3.5–5 × 10^6 irradiated, fractionated cells/culture. The results are presented in Table X. While the degree of reconstitution is not as great as that obtained with cultured spleen cells, it is clear that significant reconstitution is provided by cells with sedimentation velocities greater than...
TABLE IX
Reconstitution with Irradiated 3 Day-Cultured Spleen Cells

| Spleen cells | Irradiated 3 day-cultured spleen cells | PFC (% of control) |
|--------------|--------------------------------------|--------------------|
| Treatment*   | Cells/culture | Treatment | Cells/culture | Exp. 1 | Exp. 2 |
| ---          | $15 \times 10^6$ | --- | $0$ | 100 (83-121)$§$ | 100 (90-111)$|| |
| 7S          | $15 \times 10^6$ | --- | $7.5 \times 10^6$ | 19 (17-22) | 24 (20-29) |
| 7S          | $15 \times 10^6$ | --- | $7.5 \times 10^6$ | 78 (66-91) | 88 (78-99) |
| ---          | $15 \times 10^6$ | --- | $10^6$ | 123 (119-128) | 99 (69-141) |

Exp. 3
| $15 \times 10^6$ | --- | $0$ | 100 (84-119)$¶$ |
| 7S          | $15 \times 10^6$ | --- | $0$ | 8 (5-11) |
| 7S          | $15 \times 10^6$ | --- | $5 \times 10^6$ | 48 (35-66) |
| ---          | $15 \times 10^6$ | --- | $5 \times 10^6$ | 66 (54-81) |
| 7S          | $5 \times 10^6$ | 7S | $5 \times 10^6$ | 8 (5-11) |
| ---          | $15 \times 10^6$ | 7S | $5 \times 10^6$ | 52 (40-67) |

* 7S treatment of spleen cells as usual. Titers for treatment: exp. 1, 2,900; exp. 2, 1,250; exp. 3, 1,000. 7S antibody, C3B6F1.
† Spleen cells were cultured in Marbrook chambers for 3 days in the absence of antigen. The cells were then irradiated (1,000 rad) and combined with untreated or 7S-treated spleen cells as shown in the table. For exp. 3, a portion of the spleen cells was treated with 7S antibody before being cultured for 3 days. These cells provided 7S-treated, irradiated, 3 day-cultured cells.
§ All cultures contained $10^6$ SRBC. Control value, 1,500 PFC/culture. (Cells, C3B6F1.)
¶ Control value, 1,800 PFC/culture. (Cells, DBA.)
 fft Control value, 1,200 PFC/culture. (Cells, DBA.)

4.5 mm/h. This result supports our interpretation that reconstitution is effected by A cells.

Mechanism of the Effect.—The results presented above indicate that treatment of spleen cells with 7S antibody results in an alteration of the A cell population. This could be the result of either an intrinsic alteration of the A cells by exposure to 7S antibody, or, alternatively, the formation of a stable association between 7S antibody and these cells. Since the effect is antigen specific, while A cells are apparently not antigen restricted (23), the latter mechanism is inferred.

In order to test whether 7S antibody becomes cell associated during incubation, FITC-7S conjugates were formed as described in Materials and Methods. FITC-labeled mouse albumin was used as the control. Spleen cells were then treated in the standard way with the labeled preparations. The labeled 7S was used at a concentration of 0.14 mg/ml (ascites derived, titer at 0.14 mg/ml = 2,000), the labeled albumin, at a concentration of 0.06 mg/ml. Assuming a molecular weight of 160,000 for 7S antibody and 70,000 for albumin, this means that approximately the same number of labeled molecules were present in both
Fig. 4. Sedimentation analysis of reconstituting cells. 3 day-cultured spleen cells (see text) were separated by velocity sedimentation. Fractions were combined to form four pools (containing cells with sedimentation velocities of 2-3 mm/h, 3-4 mm/h, 4-5 mm/h, and 5-6 mm/h) and each pool of cells was then irradiated (1,000 rad). Upper graph: sedimentation profile of 3 day-cultured spleen cells. O—O, total cells; C—C, nucleated cells. Lower graph: response obtained from $15 \times 10^6$ 7S-treated spleen cells cultured alone, or combined with $1.5 \times 10^6$ irradiated fractionated cells from each of the pools described above. Response represented as GM ± SE as percent of control. Control value, provided by $15 \times 10^6$ untreated spleen cells, was 850 PFC/culture. Cells, DBA; 7S antibody, C3B6F1. (7S treatment of spleen cells was carried out at a titer of 1,500.)

incubation mixtures. After treatment and washing, the spleen cells were scored for percent fluorescing cells, and the results are shown in Table XI. In the 7S-treated group, 1% of total cells (~2% of nucleated cells) became labeled, while in the albumin-treated group just 0.03% of total cells (0.07% of nucleated cells) became labeled. Thus, treatment with 7S resulted in a specific association of 7S with a small proportion of cells.

It is not clear from the data presented whether 7S becomes associated with a small proportion of cells of all classes, or specifically with a larger proportion of a distinct class of cells. To clarify this point, cells were incubated with FITC-conjugated 7S as described above and then subjected to sedimentation analysis. After sedimentation, each fraction was scored for cell number and percent fluorescing cells. The results are shown in Fig. 5, with both total fluorescent cells per fraction and percent fluorescent cells per fraction being plotted vs.
TABLE X

Reconstitution with Irradiated Spleen Cell Fractions

| Treatment | Cells/culture | Sedimentation velocity | PFC (% of control) |
|-----------|--------------|------------------------|--------------------|
| _          | _            | mm/h                   | Cells/culture      |
| _          | _            | 0                      | 100 (96-105)       |
| 7S§        | 15 × 10^6   | 2.5-3.5                | 5 × 10^6           | 25 (19-33) |
| 7S         | 15 × 10^6   | 3.5-4.5                | 5 × 10^6           | 21 (19-24) |
| 7S         | 15 × 10^6   | 4.5-6.0                | 3.5 × 10^6         | 48 (39-59) |

* Normal spleen cells were separated by velocity sedimentation as described in Materials and Methods. Fractions were combined to form three pools (containing cells with sedimentation velocities of 2.5-3.5 mm/h, 3.5-4.5 mm/h, and 4.5-6.0 mm/h) and each pool was irradiated (1,000 rad). Irradiated cells from each pool were then combined with 7S-treated spleen cells as shown in the table.

† All cultures contained 10^6 SRBC. Control value, 950 PFC/culture. (Cells, C3B6F1.)

§ Spleen cells treated with anti-SRBC 7S antibody in the usual manner, at a titer of 1,500. (7S antibody, C3B6F1.)

TABLE XI

Association of FITC-Conjugated 7S Antibody with Spleen Cells

| Spleen cell treatment | Total cells counted | Fluorescent* cells | % fluorescent cells |
|-----------------------|---------------------|--------------------|---------------------|
| FITC-7S‡              | 2,500               | 25                 | 1.0                 |
| FITC-albumin§          | 8,300               | 3                  | 0.035               |

* Fluorescent cells were scored using a Zeiss Universal microscope with fluorescence attachment (Carl Zeiss, Inc., New York).

† Spleen cells were treated, in the usual manner, with FITC-labeled anti-SRBC 7S antibody at a concentration of 0.14 mg/ml. (7S antibody, C3B6F1; cells, DBA).

§ Spleen cells were treated with FITC-labeled mouse albumin at a concentration of 0.06 mg/ml. This concentration is equivalent, on a molar basis, to 7S antibody at a concentration of 0.14 mg/ml. Both the FITC-7S and FITC-albumin preparations have a fluorescein to protein ratio of 1.4:1 (see Materials and Methods).

sedimentation velocity. The maximum enrichment is obtained at 6 mm/h; the distribution of total number of fluorescent cells is very similar to the A cell profile found by Gorczynski et al. (23). This finding demonstrates that 7S antibody becomes associated with a distinct class of cells and is compatible with this class being the A cell population.

Inhibitory Effect of Intact 7S Antibody and F(ab')2 Fragments.—If the ability of 7S antibody to alter the A cell population is indeed the result of its association with this cell, it might be expected that intact 7S molecules, but not F(ab')2 fragments, will alter the ability of a spleen cell population to mount an immune response, since it is known that the cytophilic property of 7S antibody resides in the Fc portion of the molecule (31).
S. ABRAHAMS, R. A. PHILLIPS, AND R. G. MILLER

Fig. 5. Sedimentation analysis of cells which become associated with 7S antibody. Spleen cells were treated with FITC-conjugated 7S antibody and then separated by velocity sedimentation. Upper graph: •—•, total cells per fraction; O—O, nucleated cells per fraction. Lower graph: •—•, total fluorescent cells per fraction; X—X, percent fluorescent cells per fraction. Percent fluorescent cells of unfractionated spleen (nucleated) = 1.5%. Cells, DBA; 7S antibody, C3B6F1. (Treatment of spleen cells with FITC-7S antibody was carried out at a concentration of 0.14 mg/ml; titer of FITC-7S at 0.14 mg/ml is 2,000.)

F(ab')2 fragments were very kindly prepared by Dr. N. R. St. C. Sinclair as previously described (22). Spleen cells were treated as usual with equivalent concentrations of intact anti-SRBC 7S antibody or F(ab')2 fragments prepared from the same sample of 7S. Equivalence was based on hemagglutination titers, since the hemagglutinating efficiency of F(ab')2 is comparable to that for the intact molecule (22). The results are shown in Table XII. Treatment with intact 7S antibody, but not with the F(ab')2 fragments, reduces the immunocompetence of the spleen cells. This difference in properties of the two molecules indicates that the effect of 7S antibody on the immunocompetence of A cells is a result of the association of 7S antibody with this cell, and that the association is mediated by an Fc-binding site on the A cell.

DISCUSSION

We have shown that exposure of spleen cells to specific 7S antibody, in the absence of antigen, results in a functional depletion of A cells with respect to
MECHANISM OF 7S INHIBITION

TABLE XII

| Spleen cell treatment                  | Hemagglutination titer | PFC (% of control) |
|---------------------------------------|------------------------|--------------------|
|                                       |                        | Exp. 1*            |
|                                       |                        | Exp. 2†            |
|                                        |                        |                    |
| —                                     | —                      | 100 (95–105)       |
| 7S§                                   | 125                    | 28 (24–33)         |
| 7S‖                                   | 125                    | 29 (26–32)         |
| F(ab')₂¶                              | 125                    | 109 (95–125)       |

* All cultures contained 15 × 10⁶ spleen cells and 10⁶ SRBC. Control value, 1,500 PFC/culture. (Cells, DBA.)
† Control value, 2,000 PFC/culture. (Cells, DBA.)
§ Untreated anti-SRBC antibody (C3H). A hemagglutination titer of 125 is equivalent to a facilitated hemolytic titer of 2,000.
‖ Same sample of 7S antibody, incubated with inactive pepsin (control for F(ab')₂ preparation).
¶ Same sample of 7S antibody, digested with pepsin (see Materials and Methods).

The antigen against which the antibody carries its specificity. This effect appears to be the result of an association between A cells and 7S antibody, mediated by an Fc receptor site.

A necessary early step in the induction of an immune response to an intact erythrocyte antigen is the interaction of A cells (macrophages) and antigen, presumably resulting in the presentation of the antigen, in an immunogenic form, to the lymphoid compartment (32–34). Our results suggest that previous association of specific 7S antibody with A cells alters the subsequent A cell-antigen interaction in such a way that antigen is not presented to the lymphoid compartment in an immunogenic form. Presumably, previous association of 7S antibody with antigen would similarly alter the subsequent A cell-antigen interaction, and our experiments with antigen-antibody complexes are compatible with this prediction (6).

The importance of this mechanism with respect to normal feedback control of an in vivo immune response is at present a matter of speculation. It has been shown that SRBC-bearing macrophages from normal but not from 7S antibody-treated donors induce a response in normal recipients (5, 35, 36), which implies that 7S antibody can alter the interaction of A cells and erythrocytes in vivo as well as in vitro. Furthermore, it has been shown that inhibition of the immune response to SRBC in vivo is accomplished far more efficiently by intact 7S antibody than by F(ab')₂ fragments (37). This observation is compatible with the proposed mechanism playing a significant role in vivo.

During the course of a normal immune response, antigen, 7S antibody, and A cells would all be present. Under these conditions, the 7S antibody could either become associated with A cells (via the Fc portion of the molecule) or, alternatively, form complexes with the antigen (via the Fab portion). In either case, the
subsequent interaction of A cells and antigen, which normally results in the
presentation of immunogenic antigen to the lymphoid compartment, would be
altered by the presence of 7S antibody. This model is compatible with the feed-
back model of Bystryn et al. (38), and identifies the immunogen-containing
compartment as the A cell.

While we have demonstrated that specific antierythrocyte 7S antibody can
inhibit the response to erythrocytes by altering the interaction of A cells and
erythrocytes, this certainly does not rule out the possibility that other mecha-
nisms may operate under different conditions. Diener and Feldmann (11)
have clearly demonstrated that two separate mechanisms, both different from
that postulated above, are involved in antibody-mediated inhibition of an
A cell-independent antigen. These two mechanisms are (a) antigen neutraliza-
tion by saturation of antigenic determinants, and (b) inactivation of B cells as a
result of an interaction of antigen and antibody with B cell receptors. Sinclair
and Chan (12) have postulated that 7S antibody can act by “turning off”
lymphoid cells after a response has been induced. MacDonald et al. (33) have
shown that, in the presence of anti-SRBC 7S antibody, destruction of SRBC, mediated
by polymorphonuclear leukocytes, can occur. Thus, 7S antibody-mediated
immunoregulation may represent a complex homeostatic control system in-
volving several distinct mechanisms.

SUMMARY

A cell culture system was used to investigate the mechanism of action of the
feedback inhibition caused by specific 7S antibody. It was found that preincu-
bation of spleen cells with specific 7S antibody led to a marked reduction in the
in vitro response of the treated spleen cells to the antigen used to prepare the
antibody. The inhibition was not caused by a carry-over of free antibody nor
by the release of 7S antibody from the cells. Rather, the preincubation appeared
to specifically inactivate one of the cells required for initiation of an in vitro
response. Since the suppression could be reversed by addition of untreated cells,
it was possible to characterize the properties of the reconstituting cell. This cell
is identified as the nonlymphoid accessory cell (A cell) by several criteria. (a)
Suppression can be demonstrated only in assay systems requiring functional
A cells. (b) The most active sources for reconstitution are also good sources for
A cells. (c) The sedimentation velocity of the reconstituting cell is identical with
that for A cells. (d) Like A cells, the reconstituting cell is resistant to high
doses of ionizing radiation. (e) The reconstituting ability is not affected by
anti-0 antibody. Of the three cells required for the initiation of an immune
response, A cells, bone marrow-derived cells, and thymus-derived cells, the
data are only compatible with the reconstituting cell being an A cell.

Additional experiments suggest that the Fc portion of 7S antibody binds to
the surface of A cells. Thus, fluorescein isothiocyanate-labeled 7S antibody
binds specifically to cells with properties similar to those described above, and
MECHANISM OF 7S INHIBITION

F(\(ab'\))\(_2\) fragments, lacking Fc portion, are unable to cause immunosuppression when they are preincubated with spleen cells. It is possible that this binding is related to the specific suppression caused by 7S antibody molecules.

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