ANTIGEN-DEPENDENT IgM-MEDIATED ENHANCEMENT OF THE SHEEP ERYTHROCYTE RESPONSE IN MICE

Evidence for Induction of B Cells with Specificities Other Than That of the Injected Antibodies

BY BIRGITTA HEYMAN, GIANCARLO ANDRIGHETTO, AND HANS WIGZELL

From the Department of Immunology, Biomedicum, S-751 23 Uppsala, Sweden

Regulation of the immune system is vital to efficient functioning. Key factors in this regulation are the two specific elements in the system, the immunogens and the antigen-specific antibody molecules and receptors. Administration of antibodies to the immune system in vivo may have quite paradoxical consequences depending on the circumstances of administration. Initially, it was found that, whereas immune serum would normally reduce the humoral response against the relevant antigen if administered early enough after immunization (1, 2), administration of antibodies could, with regard to induction of delayed-type hypersensitivity reactions, frequently lead to an enhanced induction of this form of immune reaction (3). Dissection of antibodies into IgM and IgG classes subsequently revealed that IgM would normally potentiate the immune responses against low doses of relevant antigen (4), whereas antibodies of IgG class would normally act in a selective, inhibitory manner (4, 5). The ability of IgM antibodies to exert their immunostimulatory capacity for humoral antibody production is now known to require immunocompetent T lymphocytes (6–8), and it may well be that the acute potentiating power of administered IgM antibodies is exerted at the level of T cell help for antibody production.

The capacity of the IgM antibodies to potentiate antibody production has been reported to occur through several mechanisms. One way through which IgM antibodies may function has been reported to involve idiotypic determinants, as the injection of IgM antibodies alone caused induction of synthesis of IgM antibodies with the same specificity (9). The same group of workers (8) have also found that some IgM antibodies may also have an inherent capacity to induce polyclonal B cell activation in vivo.

Other studies emphasize the features of the constant regions. IgM antibodies reactive with one antigenic determinant on an erythrocyte can thus enhance the humoral response against an entirely unrelated epitope if the latter is present on the same erythrocyte during the potentiation process (10–12). In the present study we have used monoclonal or polyclonal IgM anti-sheep erythrocyte (SRBC) antibodies with defined differences in their antigen-combining specificity to analyze the conditions under which IgM antibodies may potentiate the humoral antibody response in...
acute systems, that is, when the time of inoculation between antibodies and antigen is a matter of minutes. Our results do clearly show that binding of IgM antibodies to one group of determinants on an erythrocyte will lead to enhanced reactivity against other determinants present on the same erythrocyte (RBC).

We failed to find any evidence of an inherent ability of either monoclonal or polyclonal IgM antibodies to induce antibody production of the same specificity in the absence of administered antigen. Nor did we find any evidence that the specificity of the administered IgM antibodies would be reflected as an increased fraction of plaque-forming cells (PFC) with similar fine antigen-binding specificity appearing in these acute, potentiating systems. The implications of these results will be discussed.

Materials and Methods

Animals. Unless otherwise indicated, data were obtained with CBA/H mice, aged 12–16 wk, from the breeding facilities of Anticimex, Stockholm, Sweden, or from our own animal colony. AKR mice 10–18 wk old from our breeding were used in one experiment.

Monoclonal IgM. IgM-anti-SRBC antibody-secreting hybridomas were obtained from a fusion between spleen cells from a CBA/H mouse immunized with $4 \times 10^8$ SRBC 3 d before fusion, and the BALB/c nonsecreting hybridoma line Sp 2/0 (13). Fusion was done as described (14). In brief, the two cell types were mixed with polyethylene glycol, and seeded into microtiter dishes in selective hypoxanthine, aminopterin, and thymidine (HAT) medium. Clones with ability to lyse SRBC in presence of complement were selected, recloned by limiting dilution, and kept growing in Dulbecco's modified essential medium (DMEM) containing 5% fetal calf serum. Supernatants were collected, and the antibody class was determined by class-specific antisera and also by Sepharose CL-6B (Pharmacia Inc., Uppsala) fractionation, testing if the hemolytic capacity eluted in the 19 S or 7 S peak. Before using these antibodies in some experiments they were fractionated on a Sepharose CL-6B column. The 19 S peak containing the lytic activity was concentrated by negative pressure, dialyzed against phosphate-buffered saline (PBS), and stored at −20°C. In other experiments culture supernatants were used directly or after ammonium sulphate precipitation followed by extensive dialysis. All hybridomas used in the present study were of IgM type.

Serum IgM. CBA/H mice were injected with $4 \times 10^8$ SRBC intraperitoneally and bled on day 5. The immune serum was fractionated on a Sepharose CL-6B column, concentrated, and stored in the same way as the monoclonal antibodies.

Antigens. Goat and horse erythrocytes (GRBC and HRBC) were purchased from the National Veterinary Institute, Stockholm, Sweden, and SRBC were donated by the Department of Pathology, University of Uppsala. The blood was stored in sterile Alsevers solution at 4°C.

SRBC Surface Glycoprotein Preparation. Glycoprotein from the membranes of SRBC was prepared as described by Hamaguchi and Cleve (15). In brief, the cells were hemolyzed and centrifuged. The ghosts obtained by this procedure were mechanically homogenized and subsequently diluted 1:9 in a CHCl₃/CH₃OH mixture and centrifuged at 1,500 rpm for 10 min. The supernatant was removed and further centrifuged at 100,000 g for 60 min. This supernatant was clear, contained glycoproteins, and was used in the inhibition assay.

Antibody Titration. The reciprocal of the highest antibody dilution able to completely lyse (in presence of guinea pig complement diluted 1:200) or agglutinate a 0.25% solution of erythrocytes (all dilutions in balanced salt solution [BSS]) after 1 h incubation at 37°C was defined as the antibody titer.

Inhibition Assay. Hemolytic assays were performed as described above but a constant amount of SRBC membrane glycoprotein preparation was also added to each antibody dilution. Only BSS was added to controls. The titers were defined as for hemolysis assay described above.

Absorption Studies. Twofold serial dilutions of 100 μl of each supernatant were made in microtiter plates. Packed RBC were added so that a final erythrocyte concentration of 12.5% was obtained in each well. The plates were incubated for 1 h at room temperature while shaking gently. RBC were then spun down at 1,500 g for 10 min. 50 μl of the supernatant in
ENHANCEMENT OF THE SHEEP ERYTHROCYTE RESPONSE IN MICE

each well was transferred to individual wells in a new plate and tested in a hemolysis assay, as described above, by adding guinea pig complement and RBC to each well.

**Immunizations.** Unless otherwise indicated, groups of four to five mice were given 0.1 ml of the antibody preparations in their tail veins 1-2 h before the intravenous injection of $4 \times 10^5$ RBC in 0.1 ml PBS. Normally the mice were killed 5 d later and their spleens tested in plaque assays. Control groups received 0.1 ml of PBS or cell culture medium, containing the same amount of fetal calf serum as the injected IgM-containing supernatants, before the erythrocytes.

**Plaque Assays.** A modified version of the Jerne plaque assay (16) was used: 25 µl of a 25% RBC suspension, 100 µl of an appropriately diluted lymphocyte suspension, and 25 µl guinea pig complement diluted 1:4 were added to 0.5% agar at 45°C (300 µl Difco agar containing 0.75 mg/ml DEAE dextran [Pharmacia]), mixed, and plated on a plastic petri dish (Diam 9 cm). All dilutions were made in BSS. The plates were incubated for 2 h at 37°C.

For measuring indirect plaques 25 µl of a rabbit-anti-mouse immunoglobulin antiserum was used as developing antiserum. The number of indirect plaques is given after the direct PFC number has been subtracted. The plaques were always counted "blindly".

**Calculations.** The plaque numbers are given as the log and geometrical mean ± standard error of the mean of groups of four to five mice. The significance of the differences between the groups was tested by Students t test (17). P values >0.2 were designated not significant in the tables.

## Results

**Characteristics of the Monoclonal IgM-Anti-SRBC Antibodies.** Three CBA anti-SRBC IgM monoclonals were obtained from the same fusion. They were tested for reactivity against SRBC, HRBC, and GRBC using hemagglutination or hemolytic assays. Furthermore, the antibodies were tested for reactivity against surface glycoproteins on SRBC using a hemolysis inhibition assay. The results obtained are shown in Table I and demonstrate that the three monoclonal antibodies have different antigen-binding specificities. Unlike the other two, 16-3 does not react with GRBC, and 7-3 is the only monoclonal with reactivity towards SRBC-derived glycoproteins. The finding that 16-3 is unreactive with GRBC using hemagglutination or hemolytic assays was then further substantiated using absorption experiments as summarized in Table II. Here we also included HRBC as another antigen to be tested by absorption. HRBC constitute an antigen normally claimed to only cross-react with SRBC at the T but not the B cell level to any significant degree (18, 19). In line with this we failed

### Table I

**The Antigen-binding Specificity of the Monoclonal Antibodies**

| Antibody | Anti-SRBC | Anti-GRBC | Anti-HRBC | Anti-SRBC$^\dagger$ |
|----------|-----------|-----------|-----------|---------------------|
|          | Hit*      | Hat$^\ddagger$ | Hat | Hit | Hat | No glyco-protein | Glyco-protein added |
| 7-3      | 4096      | 1024      | 4096      | <4 | <4 | 2560 | <5  |
| 10-21    | 1024      | <4        | 2048      | <4 | <4 | 320  | 320 |
| 16-3     | 4096      | 512       | <4        | <4 | <4 | 640  | 640 |

* Reciprocal of hemolytic titer of the antibody preparations.
$^\dagger$ Reciprocal of hemagglutination titer of the antibody preparations.
$^\ddagger$ Antibody titrations before and after the addition of a competing antigen (SRBC membrane glycoprotein) to the antibody dilutions. These data were obtained with different antibody preparations than those used in the first part of this table, thus explaining the difference in titers.
**Table II A**

*Antibody 16-3 is Unable Both to Bind to and to Lyse GRBC*

| Antibody | Anti-GRBC hemolysis | Anti-SRBC hemolysis |
|----------|---------------------|---------------------|
|          | Unabsorbed | Absorbed | Percent absorbed | Unabsorbed | Absorbed | Percent absorbed |
| 10-21‡‡ | 512 | 32 | 94 | 32 | 94 | 128 | 32 | 75 | 32 | 75 |
| 50-2‡‡ | 256 | 16 | 94 | 16 | 94 | 64 | 16 | 75 | 8 | 88 |
| 7-3‡‡ | 4096 | 8 | 99.8 | <4 | >99.9 | 4096 | 8 | 99.8 | <4 | >99.9 |
| 16-3‡‡ | <4 | <4 | -- | <4 | -- | 4096 | 4096 | 0 | <4 | >99.9 |
| anti-SRBC§ | 256 | <4 | >98 | <4 | >98 | 1024 | 128 | 88 | 16 | 98 |

* Expressed as reciprocal of hemolytic titer.
‡ Culture supernatants.
§ Serum from CBA/H mice immunized with SRBC.
¶ Not tested.

**Table II B**

*Antibodies 10-21, 50-2, 7-3, and 16-3 Are Unable Both to Bind to and to Lyse HRBC*

| Antibody | Anti-HRBC hemolysis | Anti-SRBC hemolysis |
|----------|---------------------|---------------------|
|          | Unabsorbed | Absorbed | Percent absorbed | Unabsorbed | Absorbed | Percent absorbed |
| 10-21‡‡ | <4 | NT || 1024 | 1024 | 0 |
| 50-2‡‡ | <4 | NT | 1024 | 1024 | 0 |
| 7-3‡‡ | <4 | NT | 4096 | 4096 | 0 |
| 16-3‡‡ | <4 | NT | 32768 | 32768 | 0 |
| Anti-HRBC¶ | 256 | <4 | >98 | NT | NT |

* Expressed as reciprocal of hemolytic titer.
‡ Culture supernatants.
§ Serum from CBA/H mice immunized with SRBC.
¶ Not tested.

...to absorb away any anti-SRBC activity using HRBC as absorbing agent. Accordingly, as judged by binding assays of various kinds, none of our three monoclonals bind to HRBC, two out of three react with GRBC and all bind well to SRBC. However, note that 10-21 in its reaction with SRBC was distinct from the other two monoclonals in its ability to lyse SRBC while failing to agglutinate the same erythrocytes. It is thus clear that use of the above monoclonals with their distinct antigen-binding specificities should allow an analysis of the impact of the constant vs. variable regions of IgM antibodies in the enhancement of the immune response against SRBC.

**Equal Ability of Different Monoclonal and Polyclonal IgM-Anti-SRBC Antibodies to Enhance Anti-SRBC Antibody Responses When Given Together with Low Doses of SRBC**

Having defined the fine specificity variation of the three IgM-anti-SRBC monoclonal antibodies, we next assessed their ability to enhance anti-SRBC antibody responses when given with low doses of SRBC in accordance with previous protocols (4). Using a constant low dose of antigen and varying amounts of antibodies we could demonstrate that all three monoclonals and the polyclonal anti-SRBC IgM serum antibodies, measured by hemolytic titers, had a similar ability to potentiate anti-SRBC PFC production (see Fig. 1 for a representative experiment out of three performed). At the present dose of $4 \times 10^5$ SRBC, the optimal dose of antibodies as estimated by...
998 ENHANCEMENT OF THE SHEEP ERYTHROCYTE RESPONSE IN MICE

Fig. 1. Dose-response diagrams of mice given different Sepharose-purified monoclonal or serum IgM-antibodies with a titer of 20 (▲), 200 (●), or 2048 (■), or PBS (□) before 4 × 10⁵ SRBC. Standard errors are indicated as vertical bars.

hemolytic titer was reached at dilution 1:200 using 0.1 ml vol for injection. Higher amounts of monoclonal antibodies failed to further increase the enhancement, and in most experiments this was also the case for polyclonal serum IgM-anti-SRBC antibodies.

This means that there seemingly exists an optimal amount of IgM per erythrocyte to obtain maximum enhancement of the anti-RBC antibody response. This increase as measured by quantity of the response (here numbers of PFC) would seem to be of the same magnitude regardless of the fine variations in the antigen-binding area of the IgM molecules used for induction of enhancement. Further evidence for optimal amounts IgM on the SRBC being responsible for the enhancing properties, rather than numbers or types of idiotopes present on the respective IgM antibodies, was obtained from experiments comparing the enhancing capacities of monoclonal antibodies alone with those of mixtures of monoclons of equal titers or the use of polyclonal IgM anti-SRBC serum antibodies. These experiments as exemplified in Table III demonstrate equal capacity of monoclonal IgM anti-SRBC when compared with mixtures of monoclonals or polyclonal serum IgM antibodies, to enhance the anti-SRBC response induced by low doses of SRBC. No consistent superiority of any of these reagents (or mixture of reagents) were thus noted in these assays.

Kinetics of the Enhancing Capacity of Monoclonal IgM Anti-SRBC for Anti-SRBC Responses. Reports have been published indicating that IgM antibodies against SRBC by themselves may function as polyclonal B cell activators (8). This required us to include two sets of controls, one to analyze the kinetics of enhancement observed in the present system, and another to study the specificity of antibody synthesis induced with or without antigen.

In our analysis of the kinetics of enhancement, we demonstrated that mice tested 24 h after injection with 4 × 10⁵ SRBC with or without IgM-anti-SRBC antibodies showed background numbers of PFC. Subsequently, a clear-cut rise in PFC in the animals receiving antigen plus antibodies was noted when compared with the figures obtained in mice receiving SRBC alone (see Fig. 2). IgM-anti-SRBC antibodies could
### Table III

*The Degree of Enhancement by Different Monoclonal Antibodies and Serum IgM in Relation to Antibody Concentration and/or Number of Clones*

| Experiment | Injection I | Htt* | Hat‡ | Injection II | PFC-anti-SRBC§ | P (vs. control) | P (vs. mix) | P (vs. IgM) |
|------------|-------------|------|------|-------------|----------------|----------------|-------------|------------|
| 1          | 7-3††       | 128  | 16   | 4 × 10⁵ SRBC | 4.99 ± 0.024   | <0.001         | NS¶         |
|            | (96.942)    |      |      |             | (95.898)       |                |             |            |
| 10-21††    | 128         | <4   | 4 × 10⁵ SRBC | 5.04 ± 0.087   | <0.005         | NS            |
|            | (116.066)   |      |      |             | (111.488)      |                |             |            |
| 16-3††     | 128         | 16   | 4 × 10⁵ SRBC | 4.98 ± 0.060   | <0.005         | NS            |
| Mix**      | 256         | 32   | 4 × 10⁵ SRBC | 5.06 ± 0.060   | <0.001         |
|            | (116.066)   |      |      |             | (126.066)      |                |             |            |
| DMEM       |             | 4 × 10⁵ SRBC | 4.13 ± 0.17 |             |
|            | (13.493)    |      |      |             | (13.493)       |                |             |            |
|            |             |      |      |             | (138.038)      |                |             |            |
| 16-3††     | 128         | NT‡‡ | 4 × 10⁵ SRBC | 5.14 ± 0.043   | <0.001         | <0.001        |
| IgM§§      | 128         | NT   | 4 × 10⁵ SRBC | 4.85 ± 0.027   | <0.005         |
| PBS        |             | 4 × 10⁵ SRBC | 4.19 ± 0.16 |             |
|            | (70.795)    |      |      |             | (70.795)       |                |             |            |

* Reciprocal of direct hemolytic titer.
‡ Reciprocal of direct hemagglutination titer.
§ Log PFC/spleen. Figures within brackets equal geometrical mean.
¶ 19 S fraction of culture supernatants.
† For simplicity, P values > 0.2 are denoted not significant.
** Equal amounts of the individual monoclonal antibody preparations tested in this experiment were mixed and 0.1 ml of the mixture was injected.
‡‡ Not tested.
§§ 19 S fraction of serum from CBA/H mice immunized with SRBC.

Also be shown to potentiate the IgG-anti-SRBC response as demonstrated in Table IV. Using a somewhat higher antigen dose (4 × 10⁶) a significant increase of IgG-anti-SRBC PFC was observed in the present IgM potentiated system.

From this we can conclude that the enhancement of antibody synthesis by IgM anti-SRBC antibodies is not confused by errors, such as false positive PFC induced by the injected antibodies passively adsorbed to cells, but rather represents an active increase in the synthesis of anti-SRBC antibodies, including Ig classes of types other than the enhancing molecules. The parallel kinetics of the curves between the PFC values in mice receiving SRBC alone or in addition anti-SRBC antibodies would indicate an increased efficiency in a normal step of anti-SRBC induction, rather than the introduction of some new mechanism.

**Failure of Monoclonal or Polyclonal IgM-Anti-SRBC Antibodies to Induce an Active Anti-SRBC Response in the Absence of SRBC.** To further analyze the possibility that IgM-anti-SRBC antibodies by themselves may cause production of IgM-anti-SRBC antibodies without addition of any antigen (9), we carried out a very extensive study on the possible impact of giving IgM anti-SRBC antibodies alone under varying conditions. To make this study as broad as possible, we examined our three monoclonal IgM separately, as a mixture of all three, or tried polyclonal IgM anti-SRBC serum.
1000 ENHANCEMENT OF THE SHEEP ERYTHROCYTE RESPONSE IN MICE

Fig. 2. Kinetics of the direct PFC response of mice given Sepharose-purified monoclonal antibody 10-21 with a hemolytic titer of 256 (■) or PBS (○) followed by 4 × 10⁷ SRBC. Standard errors are indicated as vertical bars. The P value of the difference between control and experimental groups was, except on day 1, <0.001.

TABLE IV

| Injection I | Ht* | Injection II | Direct PFC- anti-SRBC‡ | P (vs. control) | Indirect PFC- anti-SRBC§ | P (vs. control) |
|-------------|-----|--------------|------------------------|----------------|--------------------------|----------------|
| 7.3 | 1024 | 4 × 10⁶ SRBC | 4.76 ± 0.035 | NS† | 5.49 ± 0.097 | <0.005 |
| PBS | | 4 × 10⁶ SRBC | 4.74 ± 0.063 | 4.77 ± 0.11 | | |

* Reciprocal of direct hemolytic titer.
‡ Log direct PFC/spleen ± SE; figures within brackets equal geometrical mean.
§ Log indirect PFC/spleen ± SE; figures within brackets equal geometrical mean.
‖ Ammonium-sulphate-precipitated culture supernatant.
† Not significant (P > 0.2).

antibodies as well. A total of 21 experiments of this kind were performed with very similar, negative results: no ability of IgM anti-SRBC antibodies to alone induce an active anti-SRBC response was observed. In Table V we have included three experiments to represent the various approaches used as to reagents, titers of IgM antibodies applied, etc. Table V includes the only individual group of animals where one might have considered a positive effect at one particular titer step of IgM antibodies (experiment 1, titer 128), but note that the increase was very marginal from 57 in the background to 125 in the antibody-treated group. This, plus the fact that none of the other 20 experiments showed even this weak increase by IgM anti-SRBC antibodies, makes us feel confident that IgM-anti-SRBC antibodies can not
| Experiment 1 | Inj I | Ht* | Inj II | PFC-anti-SRBC§ | P (vs. control) |
|-------------|------|-----|--------|---------------|----------------|
| IgM§        | 1024 | 0   | 1.89 ± 0.073 (76) | NS||
| IgM         | 512  | 0   | 1.82 ± 0.073 (66) | NS |
| IgM         | 256  | 0   | 1.82 ± 0.073 (66) | NS |
| IgM         | 128  | 0   | 2.10 ± 0.062 (125) | <0.001 |
| IgM         | 64   | 0   | 1.95 ± 0.11 (89) | NS |
| PBS         | 0    | 1.76 ± 0.06 (57) | NS |
| IgM         | 256  | 4 × 10⁶ SRBC | 5.54 ± 0.072 (122.395) | <0.001 |
| PBS         | 4 × 10⁶ SRBC | 4.31 ± 0.12 (20.749) | |

| Experiment 2 | Mix¶ | 8192 | 0   | 1.76 ± 0.060 (57) | NS |
| Mix         | 1024 | 0   | 2.26 ± 0.14 (183) | NS |
| Mix         | 512  | 0   | 1.99 ± 0.14 (99) | NS |
| Mix         | 256  | 0   | 2.32 ± 0.17 (210) | NS |
| Mix         | 64   | 0   | 1.89 ± 0.12 (78) | NS |
| DMEM        | 0    | 2.0 ± 0.23 (100) | NS |
| Mix         | 1024 | 4 × 10⁶ SRBC | 3.72 ± 0.20 (5.208) | <0.05 |
| DMEM        | 4 × 10⁶ SRBC | 3.07 (1.168) | |

| Experiment 3 | 7-3** | 4096 | 0 | 4.27 ± 0.10 (18.460) | <0.1 |
| 10-21**      | 256   | 4 × 10⁶ SRBC | 4.63 ± 0.091 (42.491) | <0.01 |
| 16-3**       | 8192  | 4 × 10⁶ SRBC | 4.80 ± 0.057 (62.842) | <0.005 |
| DMEM         | 4 × 10⁶ SRBC | 3.91 ± 0.29 (5.400) | |
| 7-3**        | 4096  | 0   | 2.27 ± 0.081 (188) | NS |
| 10-21**      | 256   | 0   | 2.28 ± 0.16 (192) | NS |
| 16-3**       | 8192  | 0   | 2.04 ± 0.24 (110) | NS |
| DMEM         | 0     | 2.47 ± 0.23 (298) | NS |

* Reciprocal of hemolytic titer of the antibody preparations.
§ Log PFC/spleen ± SE; figures within brackets equal geometrical mean.
§§ 19 S fraction of serum from CBA/H mice immunized with SRBC.
|| Not significant (P > 0.2).
¶ Equal amounts of the three individual monoclonal antibody preparations were mixed, and 0.1 ml of the mixture, or a dilution in DMEM, was injected.
** Culture supernatants.
induce any further IgM-anti-SRBC antibodies by themselves in the absence of relevant antigen in the presently studied system.

The Fine Specificity of the IgM Monoclonal Anti-SRBC Antibodies is Decisive in Allowing the Induction of Potentiation of the Anti-SRBC Response, but Does Not Determine the Fine Specificity of the Enhanced Antibody Production. A crucial question in systems studying potentiation of antibody production by previous administration of antibodies is whether this enhancing ability may influence in a very direct manner the antibody specificity of the Ig molecules produced. Whereas it is clear that injection of monoclonal antibodies some days or weeks before the actual challenge with antigen may lead to either enhancement or suppression of a particular group of antibodies with similar idiotypes/fine antigen-binding features as the administered antibodies (20–22), confusion exists as to whether such an impact is seen in acute potentiation of the type studied in this article. On one hand, reports exist indicating that IgM antibodies against one epitope on an erythrocyte will nicely potentiate the response against an unrelated epitope if present on the same erythrocyte (10–12). On the other hand, the reports that IgM antibodies may, by themselves, acutely induce an active production of more antibodies with the very same specificity (9) would by necessity imply that idiotypic determinants may play a highly decisive role even in the present acute IgM potentiation systems. To analyze this question further we took advantage of the fine specificity variations in our three monoclonal IgM antibodies (all binding to SRBC, two binding to GRBC, and none reacting with HRBC).

SRBC and GRBC cross-react extensively at both B and T cell levels, whereas HRBC and SRBC have some detectable cross-reactions at the T lymphocyte level but virtually no cross-reactions at the level of humoral antibodies (18–19). First, we used GRBC instead of SRBC as the immunogen with or without IgM-anti-SRBC monoclonals, and assayed for PFC against both GRBC and SRBC. The results were clear cut: the two monoclonal antibodies, which also cross-react with GRBC, worked beautifully as potentiators of IgM PFC induction against GRBC and SRBC (see Table VI for one representative experiment out of three). On the other hand, the monoclonal IgM anti-SRBC antibody 16-3, which does not cross-react with GRBC, also failed to have any impact in these sets of experiments when GRBC was the immunogen.

In contrast, when the same non-GRBC cross-reacting monoclonal is used to potentiate the response against SRBC and GRBC now using SRBC as the immunogen, the “normal” potentiation against both SRBC and GRBC is seen (Table VI). A representative experiment showing that the same relative degree of enhancement of anti-GRBC PFC is seen, whether or not we use monoclonals cross-reacting with GRBC together with SRBC as the immunogen, is shown in Table VII. These results are thus in direct agreement with the concept that the IgM antibodies potentiate via binding to the erythrocytes, thereby allowing other epitopes on the same erythrocyte to display enhanced immunogenic properties.

Further evidence supporting this concept was then derived from studies using HRBC instead of GRBC in similar kinds of experiments. We now investigated whether anti-SRBC IgM monoclonals, when given with HRBC alone, SRBC alone, or with mixtures of the two erythrocytes, would potentiate the respective anti-HRBC and -SRBC responses. While interpreting the results from these studies, it should be remembered that none of the monoclonal IgM anti-SRBC express any binding to
TABLE VI

Failure of Monoclonal Anti-SRBC-Antibodies which Are Non-Crossreactive with GRBC to Potentiate the Anti-GRBC PFC Response When GRBC Is the Immunogen

| Inj I | Hlt* | Inj II | PFC-anti-GRBC‡ | P (vs. control) | PFC-anti-SRBC | P (vs. control) |
|-------|------|--------|----------------|----------------|---------------|----------------|
| 16-3§ | 1024 | 4 × 10⁶ GRBC | 4.04 ± 0.18 | (8.787) | 3.37 ± 0.12 | NS |
| 7-3§ | 2048 | 4 × 10⁶ GRBC | 4.32 ± 0.13 | (32.990) | 3.99 ± 0.18 | <0.025 |
| 10-21§ | 512 | 4 × 10⁹ GRBC | 4.34 ± 0.13 | (21.955) | 3.78 ± 0.12 | <0.05 |
| DMEM | 4 × 10⁹ GRBC | 3.74 ± 0.13 | (5.529) | 3.23 ± 0.17 | (1.701) |
| 16-3§ | 1024 | 4 × 10⁶ SRBC | 4.23 ± 0.085 | (17.161) | 4.72 ± 0.061 | <0.01 |
| DMEM | 4 × 10⁹ SRBC | 3.43 ± 0.17 | (2.689) | 4.17 ± 0.15 | (14.774) |

* Reciprocal of hemolytic titer.
‡ PFC numbers expressed as 1⁰log PFC/spleen ± SE; figures within brackets equal geometrical mean.
§ Culture supernatant, not purified.
¶ The only antibody not cross-reacting with GRBC.
¶ Not significant (P > 0.2).

TABLE VII

Monoclonal Antibodies Non-Crossreactive with GRBC Can Potentiate the Anti-GRBC PFC Response When SRBC Is the Immunogen

| Inj I | Hlt* | Inj II | PFC-anti-GRBC‡ | P (vs. control) | PFC-anti-SRBC | P (vs. control) |
|-------|------|--------|----------------|----------------|---------------|----------------|
| 7-3||| 4096 | 4 × 10⁹ SRBC | 4.27 ± 0.68 | (18.460) | 3.82 ± 0.15 | (6.614) |
| 10-21||| 256 | 4 × 10⁶ SRBC | 4.63 ± 0.091 | (42.491) | 4.13 ± 0.10 | (13.382) |
| 16-3¶| 8192 | 4 × 10⁶ SRBC | 4.80 ± 0.57 | (62.842) | 4.46 ± 0.063 | (29.021) |
| DMEM | 4 × 10⁶ SRBC | 3.73 ± 0.23 | (5.400) | 3.00 ± 0.13 | (990) |

* Hemolytic titer.
‡ 1⁰log PFC/spleen ± SE; figures within brackets equal geometrical mean.
§ GRBC-PFC/spleen × 100 ± SE.
¶ SRBC-PFC/spleen ± SE.
|| Culture supernatants, not purified.
¶ The only antibody preparation that is not cross-reactive with GRBC.

HRBC, but that SRBC and HRBC cross react at the T cell level (18-19). The results obtained with HRBC in the above context are presented in Table VIII. Note first that cross-reactivity at the B cell level is negligible as expected (<1% in either direction as indicated in experiments 4-7). Likewise, anti-HRBC responses were never potentiated when IgM-anti-SRBC antibodies were given either alone (experiment 7), together with HRBC (experiments 4-6), or when the antibodies were given with SRBC only (experiments 5-7). However, a striking potentiation against HRBC was
### Table VIII

Enhancement of the Anti-HRBC Response

| Injection I | Ht* | Injection II | PFC-anti-SRBC | P (vs. control) | PFC-anti-HRBC | P (vs. control) |
|-------------|-----|-------------|---------------|----------------|--------------|----------------|
| **Experiment I**<br>10-21| 256 | 4 x 10^8 SRBC* | 3.50 ± 0.10 | <0.001 | 4.15 ± 0.14 | <0.025 |
| | | 4 x 10^5 HRBC | (3.144) | | (14.059) | |
| | | 4 x 10^8 SRBC* | 2.13 ± 0.19 | | 3.50 ± 0.16 | |
| | | 4 x 10^5 HRBC | (133) | | (3.165) | |
| **Experiment II**<br>10-21| 256 | 4 x 10^8 SRBC* | 4.59 ± 0.10 | <0.005 | 4.32 ± 0.13 | <0.2 |
| | | 4 x 10^5 HRBC | (39.159) | | (20.837) | |
| | | 4 x 10^8 SRBC* | 3.96 ± 0.097 | | 3.81 ± 0.28 | |
| | | 4 x 10^5 HRBC | (9.650) | | (6.471) | |
| **Experiment III**<br>10-21| 128 | 4 x 10^8 SRBC* | 4.82 ± 0.089 | <0.05 | 4.21 ± 0.16 | <0.05 |
| | | 4 x 10^5 HRBC | (66.278) | | (16.126) | |
| | | 4 x 10^8 SRBC* | 4.20 ± 0.23 | | 3.17 ± 0.34 | |
| | | 4 x 10^5 HRBC | (15.785) | | (1.465) | |
| **Experiment IV**<br>7-3| 4096 | 4 x 10^8 SRBC* | 4.27 ± 0.10 | <0.1 | 4.46 ± 0.11 | <0.2 |
| | | 4 x 10^5 HRBC | (18.460) | | (28.910) | |
| | 10-21| 256 | 4 x 10^8 SRBC* | 4.63 ± 0.091 | <0.01 | 4.54 ± 0.89 | <0.05 |
| | | 4 x 10^5 HRBC | (42.491) | | (34.745) | |
| | 16-3| 8192 | 4 x 10^8 SRBC* | 4.80 ± 0.057 | <0.005 | 4.53 ± 0.066 | <0.025 |
| | | 4 x 10^5 HRBC | (62.842) | | (33.889) | |
| | | 4 x 10^8 SRBC* | 3.91 ± 0.29 | | 4.24 ± 0.076 | |
| | | 4 x 10^5 HRBC | (5.400) | | (17.339) | |
| **Experiment V**<br>7-3| 4096 | 4 x 10^8 HRBC | 2.27 ± 0.081 | NS** | 4.47 ± 0.058 | NS |
| | | 4 x 10^5 HRBC | (188) | | (27.963) | |
| | 10-21| 256 | 4 x 10^8 HRBC | 2.28 ± 0.16 | NS | 4.37 ± 0.18 | NS |
| | | 4 x 10^5 HRBC | (192) | | (23.228) | |
| | 16-3| 8192 | 4 x 10^8 HRBC | 2.04 ± 0.24 | NS | 4.24 ± 0.08 | <0.1 |
| | | 4 x 10^5 HRBC | (110) | | (17.393) | |
| | | 4 x 10^8 HRBC | 2.47 ± 0.23 | NS | 4.45 ± 0.11 | <0.1 |
| | | 4 x 10^5 HRBC | (296) | | (28.007) | |
| **Experiment VI**<br>7-3| 256 | 4 x 10^8 HRBC | 2.22 ± 0.316 | NS | 4.20 ± 0.16 | NS |
| | 16-3| 256 | 4 x 10^8 HRBC | 2.24 ± 0.66 | NS | 4.28 ± 0.076 | <0.2 |
| | | 4 x 10^5 HRBC | (174) | | (19.025) | |
| | | 4 x 10^8 HRBC | 2.25 ± 0.14 | NS | 4.24 ± 0.042 | <0.2 |
| | | 4 x 10^5 HRBC | (178) | | (17.391) | |
| | | 4 x 10^8 HRBC | 2.00 ± 0.13 | NS | 4.12 ± 0.076 | <0.2 |
| | | 4 x 10^5 HRBC | (100) | | (13.048) | |
| **Experiment VII**<br>10-21| 128 | 4 x 10^8 SRBC | 4.71 ± 0.044 | <0.001 | 2.48 ± 0.38 | NS |
| | | 4 x 10^5 HRBC | (51.104) | | (303) | |
| | | 4 x 10^8 SRBC | 3.93 ± 0.12 | NS | 2.79 ± 0.999 | (617) |
TABLE VIII—continued

| Injection I | Hlt* | Injection II | PFC-anti-SRBC‡ | P (vs. control) | PFC-anti-HRBC‡ | P (vs. control) |
|-------------|------|-------------|----------------|----------------|----------------|----------------|
| Experiment 6 | 7-3| 256 | 1 × 10⁴ HRBC | 2.23 ± 0.13 (168) | NS | 3.00 ± 0.13 (990) | NS |
| 10-21| 128 | 1 × 10⁷ HRBC | 2.18 ± 0.073 (152) | NS | 2.90 ± 0.21 (788) | NS |
| PBS | 256 | 1 × 10⁴ HRBC | 2.33 ± 0.11 (214) | NS | 3.16 ± 0.17 (1.43) | NS |
| 7-3| 4 × 10⁶ SRBC | 4.42 ± 0.10 (26,202) | <0.2 | 1.88 ± 0.073 (76) | NS |
| 10-21| 128 | 4 × 10⁶ SRBC | 4.57 ± 0.063 (3.562) | <0.0025 | 2.27 ± 0.18 (186) | NS |
| PBS | 4 × 10⁵ SRBC | 4.13 ± 0.13 (13.518) | NS | 2.06 ± 0.11 (115) | NS |

Experiment 7

| IgM†† | 1024 | 0 | 1.89 ± 0.073 (76) | NS | 2.04 ± 0.15 (108) | NS |
| IgM | 512 | 0 | 1.82 ± 0.073 (66) | NS | 1.88 ± 0.12 (76) | NS |
| IgM | 256 | 0 | 1.82 ± 0.073 (66) | NS | 2.0 ± 0.13 (100) | NS |
| IgM | 128 | 0 | 2.10 ± 0.062 (123) | <0.001 | 1.82 ± 0.073 (66) | NS |
| IgM | 64 | 0 | 1.95 ± 0.11 (89) | NS | 2.0 ± 0.045 (100) | NS |
| PBS | 0 | 1.76 ± 0.06 (57) | NS | 2.0 ± 0.13 (100) | NS |
| IgM†† | 256 | 4 × 10⁴ SRBC | 5.54 ± 0.072 (122.395) | <0.001 | 2.16 ± 0.14 (143) | NS |
| PBS | 4 × 10⁵ SRBC | 4.31 ± 0.12 (20.749) | NS | 2.41 ± 0.13 (255) | NS |

* Reciprocal of hemolytic titer of the antibody preparations.
‡ PFC numbers expressed as log PFC/spleen ± SE; figures within brackets equal geometrical mean.
§ This experiment was done with AKR mice.
‖ 19 S fraction of culture supernatants.
¶ Culture supernatants, not purified.
** Not significant (P > 0.2).
†† 19 S fraction of serum from CBA/H mice immunized with SRBC.

We observed if anti-SRBC IgM antibodies were administered into mice that received SRBC and HRBC (experiments 1–4). We could thus conclude that IgM-anti-SRBC antibodies with no cross-reacting properties to HRBC still can effectively potentiate the IgM response against this antigen if two requirements are fulfilled: the antigen in question (HRBC) has to be present in vivo, and so does the antigen (SRBC) with which the IgM antibodies react.

Discussion

It is by now a well-established fact that IgM antibodies, if administered with antigen of T-dependent nature, will frequently lead to an enhanced humoral antibody response against the immunogen (4, 6). This is particularly pronounced if care is taken to use proper amounts of immunogen and antibody and optimal timing between
antibody and antigen administration \((4, 6)\). Comparatively little is known about the actual underlying mechanism(s) of this IgM potentiation of antibody-production, where antibody and antigen are administered in close connection in terms of time. The potentiation requires the presence of immunocompetent T lymphocytes \((6-8)\); that is, IgM can not replace T helper cells for induction of antibody production. It is not known, however, whether the IgM antibodies potentiate the presentation of antigen to the T cells, increase the numbers of specific T helper cells, or make the actual activation of B lymphocytes more efficient by simultaneous activation of T lymphocytes. As IgM antibodies are supreme inducers of potentiation of this kind of humoral antibody response compared with the mostly negative impact by IgG antibodies \((4)\), one would deem it likely that constant region features of the IgM molecules are responsible for these differences. Speculations have been made as to a possible connection between receptors for aggregated IgM on T helper cells and the observed IgM potentiation, but they remain to be proven. In addition, IgM antibodies have been reported to sometimes display an inherent ability to induce in the absence of immunogen a highly potent antibody synthesis of IgM antibodies with the very same antigen-binding specificity as the injected antibodies \((9)\). Furthermore, polyclonal activation of B cells by IgM antibodies alone has also been reported \((8)\). This would then indicate that variations in the antigen-binding, idiotypic area of IgM antibodies may sometimes endow such an antibody with a dramatic capacity to function as a powerful and sometimes selective antibody inducer, making interpretations of earlier IgM enhancement data very difficult. Other results \((10-12)\) would indicate the constant region of IgM as the most relevant, because binding of IgM molecules to a particular group of epitopes on an antigen could be shown to lead to a significant enhancement of the humoral response against other antigenic determinants simultaneously present on the immunogen.

We have explored the ability of monoclonal or polyclonal IgM anti-SRBC antibodies in the murine system to potentiate anti-SRBC antibody production in relation to the above findings as this antigenic system represents the most studied system in this regard. Our new approach was to use well-defined monoclonal IgM anti-SRBC antibodies with different antigen-combining specificity, produced and used in the same strain of mice, CBA/H. First, we demonstrated that the ability of either monoclonal or polyclonal IgM anti-SRBC antibodies to potentiate the immune response against low doses of SRBC was very similar when compared at identical hemolytic titers. Thus, we find no evidence of variable region features contributing to potentiation in a quantitatively detectable manner. Second, using titration curves, we demonstrated that there seemingly exists an optimal amount of IgM which, when bound to the SRBC, allows maximal effect, and that the use of antibody concentrations above this titration point failed to further enhance the potentiation. We do not know, however, at what level this limitation lies. The ability of the IgM anti-SRBC antibodies to potentiate the response against low doses of SRBC displayed time kinetics in line with the view that the induction of anti-SRBC B cells was made more efficient in general, without having to consider the introduction of some new factor in the induction of antibody synthesis by the added IgM antibodies. An extensive series of experiments was then set up in various ways to explore whether IgM anti-SRBC antibodies alone could induce IgM anti-SRBC antibody production as previously reported \((9)\).
Our results using monoclonal or polyclonal IgM anti-SRBC antibodies here were uniformly negative. Furthermore, as the antibodies failed to change the background PFC against SRBC and another antigen, HRBC, no general, polyclonal B cell activation could have occurred. The fact that not only monoclonal, but also polyclonal IgM anti-SRBC antibodies failed to enhance would argue against monoclonal antibody variability being responsible for discrepancies between our results and those of others. We thus feel confident that in the present CBA/H anti-SRBC system IgM-anti-SRBC syngeneic antibody molecules fail to induce either specific IgM-anti-SRBC or polyclonal B cell activation. This would suggest that the previously reported capacity of IgM antibodies to acutely induce intense antibody production with the same specificity in the absence of antigen (9) may be restricted to certain experimental conditions.

Using monoclonal IgM-anti-SRBC that did or did not cross-react with GRBC, an antigen that extensively cross-reacts with SRBC at the epitope level, we could clearly demonstrate that the binding of IgM antibodies that do not cross-react with GRBC to epitopes on SRBC would cause the same degree of potentiation of the antibody response as cross-reacting IgM when measured against GRBC. In contrast, the same monoclonal antibodies failed altogether to potentiate either anti-SRBC or -GRBC responses when GRBC was the immunogen used. These findings confirm the results in another system (12), and are to be expected if the constant-region class-specific features of IgM molecules are the decisive factors in the IgM induced potentiation of antibody responses.

None of our anti-SRBC monoclonal antibodies were cross-reactive with HRBC as one would expect from the very low cross-reacting properties of anti-SRBC and -HRBC antibodies in general (for example, see experiment 4-7, Table VIII). In agreement with the failure to bind HRBC, none of the anti-SRBC monoclonals could enhance the anti-HRBC production if given with SRBC alone or with HRBC alone. However, a dramatic impact of enhancement was noted when SRBC and HRBC were given together with anti-SRBC antibodies, suggesting that the potentiated anti-SRBC response now could help the anti-HRBC response. The underlying mechanism of this finding is presently under scrutiny. As HRBC can cross-react with SRBC at the T cell level (18-19), one may speculate that the observed effect is specific and mediated by the potentiated induction of T helper cells for SRBC, some of which also cross-react with HRBC. These HRBC-reactive T cells could then, in the presence of HRBC, help B cells to produce anti-HRBC antibodies seen as the enhancement of HRBC-specific PFC (Table VIII, experiments 1-4). Another possible mechanism is that an increased antigen-antibody trapping in the spleen caused by the IgM (23) could subsequently lead to trapping and thereby increased immunogenicity of a second antigen, in this case HRBC (24). Experiments that will prove or disprove these assumptions, and that will more definitely define the mode of action of IgM in general in potentiating humoral antibody responses against T dependent antigens have been initiated.

**Summary**

Monoclonal or polyclonal IgM-anti-SRBC antibodies were used to enhance the anti-SRBC PFC response in mice. For potentiation to occur, the IgM antibodies must always be presented with the antigen for which they have specificity. No enhancement
of anti-SRBC response above control levels was noted with either antibodies alone or with antibodies used together with non-cross-reacting antigens.

The degree of enhancement was independent of whether only one or several different monoclonal IgM antibodies were used. Likewise, the fine specificity variation among the antibody clones failed to influence the anti-SRBC potentiation, which was shown to vary only with the amount of IgM bound to SRBC measured by hemolytic titers.

The response against epitopes on the SRBC other than those the IgM recognized was also enhanced. This was determined by injecting SRBC and a monoclonal anti-SRBC IgM that did not crossreact with GRBC into mice, and measuring the response against both antigens. Normally SRBC and GRBC cross-react at the B cell level to ~30%, and in this experiment they did so both in the control group and in the IgM group.

Using antigens that only cross-react significantly at the T cell level (SRBC and HRBC), IgM-antibodies would only enhance the anti-HRBC response if SRBC and HRBC were inoculated together. No anti-HRBC potentiation was noted when antibodies were injected alone or together with either SRBC or HRBC.

The data indicate that the constant part of the IgM molecule is of major importance in determining its enhancing properties in acute IgM-mediated potentiation of the immune responses. No evidence was obtained for a decisive role of variable regions. Furthermore, no general B cell activating properties of either mono- or polyclonal IgM-anti-SRBC antibodies could be demonstrated.

We thank Dr. D. Bellgrau for performing the hybridoma fusions and Dr. Tom Moran for reading the manuscript and correcting the English. The help of Mrs. Birgitta Ehrsson-Lagerkvist in the preparation of this manuscript is gratefully acknowledged. This work was supported by the Swedish Cancer Society.

Received for publication 1 December 1981.

References

1. Uhr, J. W., and G. Möller. 1968. Regulatory effects on the immune response. Adv. Immunol. 8:81.
2. Hoffman, M. K. 1980. Antibody regulates the cooperation of B cells with helper cells. Immunol. Rev. 49:79.
3. Uhr, J. W., S. B. Salvin, and A. M. Pappenheimer, Jr. 1957. Delayed hypersensitivity. II. Induction of hypersensitivity in guinea pigs by means of antigen-antibody complexes. J. Exp. Med. 105:11.
4. Henry, C., and N. Jerne. 1968. Competition of 19 S and 7 S antigen receptors in the regulation of the primary immune response. J. Exp. Med. 128:133.
5. Murgita, R. A., and S. I. Vas. 1972. Specific antibody-mediated effect on the immune response. Suppression and augmentation of the primary immune response in mice by different classes of antibodies. Immunology. 22:319.
6. Dennert, G. 1973. Effects of IgM on the in vivo and in vitro immune response. Proc. Soc. Exp. Biol. Med. 143:889.
7. McBride, R. A., and L. W. Schierman. 1973. Thymus dependency of antibody-mediated helper effects. J. Immunol. 110:1710.
8. Coutinho, A., and L. Forni. 1981. The enhancement of antibody response by IgM antibodies is dependent on antigen-specific T helper cells. Immunobiology. 158:182.
9. Forni, L., A. Coutinho, G. Köhler, and N. Jerne. 1980. IgM antibodies induce the production of antibodies of the same specificity. Proc. Natl. Acad. Sci. U. S. A. 77:1125.
10. Pearlman, D. S. 1967. The influence of antibodies on immunologic responses. The effect on the response to particulate antigen in the rabbit. J. Exp. Med. 126:127.
11. McBride, R. A., and L. W. Schierman. 1970. Hapten-carrier relationships of isoantigens. A model for immunological maturation based on the conversion of haptenes to carriers by antibody. J. Exp. Med. 131:377.
12. Whited-Collison, E., B. Andersson, and E. W. Lamon. 1979. Modulation of hapten-specific responses with anticanter antibody: I. Differential effects of IgM and IgG anticanter on primary direct and indirect hapten-specific plaque-forming cells. Proc. Soc. Exp. Biol. Med. 162:194.
13. Schulman, M., C. D. Wilde, and G. Köhler. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature (Lond.). 276:269.
14. McKearn, T. J., F. W. Fitch, D. E. Smilek, M. Sarmiento, and F. P. Stuart. 1979. Properties of rat anti-MHC antibodies produced by cloned rat-mouse hybridomas. Immunol. Rev. 47:91.
15. Hamaguichi, H., and H. Cleve. 1972. Solubilization and comparative analysis of mammalian erythrocyte membrane glycoproteins. Biochem. Biophys. Res. Commun. 47:459.
16. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. Science (Wash. D. C.). 140:405.
17. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 91-116.
18. Hartmann, K.-U. 1970. Induction of a hemolysin response in vitro. Interaction of cells of bone marrow origin and thymic origin. J. Exp. Med. 132:1267.
19. Vann, C., and J. Kettman. 1972. In vitro cooperation of cells of bone marrow and thymus origins in the generation of antibody-forming cells. J. Immunol. 108:73.
20. Kelsoe, G., M. Reth, and K. Rajewsky. 1980. Control of idiotope expression by monoclonal anti-idiotope antibodies. Immunol. Rev. 52:75.
21. Reth, M., G. Kelsoe, and K. Rajewsky. 1981. Idiotypic regulation by isologous monoclonal anti-idiotope antibodies. Nature (Lond.). 290:257.
22. Kelsoe, G., M. Reth, and K. Rajewsky. 1981. Control of idiotope expression by monoclonal anti-idiotope and idiotope-bearing antibody. Eur. J. Immunol. 11:418.
23. Dennert, G. 1971. The mechanism of antibody-induced stimulation and inhibition of the immune response. J. Immunol. 106:951.
24. Klaus, G. G. B., J. H. Humphrey, Annalisa Kunkl, and D. W. Donworth. 1980. The follicular dendritic cell: Its role in antigen presentation in the generation of immunological memory. Immunol. Rev. 53:3.