PRODUCTION OF A RUNTING SYNDROME AND SELECTIVE γA DEFICIENCY IN MICE BY THE ADMINISTRATION OF ANTI-HEAVY CHAIN ANTISERA*

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It is generally accepted that antigen recognition is a prerequisite for the induction of antibody formation, that recognition first occurs at the cell surface, and that the receptors involved have antibody-combining sites. By hypothesis, the specific combination of antigen with its receptor(s) results in activation of immune differentiation and subsequent synthesis of antibody, although under certain conditions specific inactivation and tolerance can ensue (1, 2). Antigen receptors located on the surface of certain lymphoid cells (3-9) are considered to be representative of the eventual cell product with regard to both specificity and immunoglobulin class (10-13). Therefore, the use of antibody directed against a particular class of heavy chain determinants displayed by antigen receptors could conceivably lead to the selective inhibition of the production of that immunoglobulin class resulting in an experimental dysgammaglobulinemia.

There are now a number of experimental findings that demonstrate suppressive effects on immunological responses as the result of exposure to anti-immunoglobulin sera including, (a) anti-allotype-induced suppression (14-16), (b) anti-idiotype-induced suppression (17, 18), (c) suppression of cell-mediated hypersensitivity and transplantation reactions in vitro (19), (d) inhibition of rosette formation (20), (e) in vivo suppression of immunoglobulin synthesis in germfree animals (21), (f) suppression of the primary plaque-forming cell (PFC) response to foreign erythrocytes in vivo (22) and in vitro (23, 24), and (g) inhibition of the cellular transfer of immunoglobulin synthesis (25). The cellular basis for many of these immunosuppressive effects is still very uncertain.

We now report findings that demonstrate that treatment of conventional Swiss mice from birth through young adulthood with antimouse γA-globulins results in a serum-γA deficiency with normal production of γM and γG and with an essentially normal complement of γA-containing cells in the gastrointestinal tract. Treatment with anti-γM-globulin preparations re-
sulted in a profound generalized suppression of γG and γA as well as γM synthesis similar to the effects reported by others (21, 22). In addition, a severe form of wasting disease resulted from anti-γM but not anti-γA and anti-γG treatment, with the dose and the time of administration of antiglobulin being critical factors in the development of this syndrome.

Materials and Methods

Animals.—Conventional Swiss mice, purchased from the Jackson Laboratory, Bar Harbor, Maine, were used in all experiments. This strain was chosen for these studies because of ease in mating and large litter size. Mouse litters were weaned on day 21.

Hemolytic Plaque Assay.—The localized hemolysis-in-gel technique was used with the modifications of Wortis and Dresser (26) to detect and enumerate cellular synthesis of γM-antibody (direct PFC). Fresh frozen pig serum diluted at 1:9 with saline served as a source of complement. Class-specific rabbit antimouse γA- and γG-immunoglobulin preparations were titrated to determine the optimal dilutions for development of indirect γA and γG PFC as previously described (27). The number of indirect PFC was calculated as the total plaques developed with facilitating antisera minus the plaques on identical plates with added complement only (direct PFC).

Hemagglutination Determination.—Sheep red blood cell (SRBC) hemagglutinins (HA) were titrated in disposable plastic plates using a Cooke microtiter (Cooke Engineering Co., Alexandria, Va.). The results were read after 2 h incubation at 37°C. Titers were expressed as log2 of the reciprocal of final dilutions showing HA observed macroscopically. The fraction of the total HA activity resistant to treatment with 0.1 M 2-mercaptoethanol (2-ME) was determined by incubation for 1 h at room temperature. Antibodies resistant to 2-ME were regarded as 7S, and those 2-ME sensitive as 19S.

Serum Immunoglobulin Levels.—Serum immunoglobulin levels were determined by the serial dilution Ouchterlony gel diffusion technique described by Arnason et al. (28). These values were reported as the reciprocal of the highest twofold dilution producing a distinct band against a commercial (Meloy Laboratories, Inc., Springfield, Va.) class-specific antiserum. Mean serum levels ± standard error for each experimental group were calculated as the numerical average of the individual values.

Antisera.—Monospecific antisera to mouse immunoglobulins were prepared in rabbits and in goats reported in detail elsewhere.3 In brief, antigen was immunoprecipitated by double diffusion in agar against specific antiserum and the precipitin bands were cut out and injected with complete Freund’s adjuvant. Three different antimouse γ-chain antisera and three different antimouse α-chain antisera were prepared. These included: rabbit antinormal mouse γM, rabbit anti-MOPC-104E tumor γM, goat antinormal mouse γM, rabbit antimouse milk γA, rabbit anti-MPC-1 myeloma γA, and goat anti-MPC-1 myeloma γA. When necessary, absorption of minor contaminants was done with newborn mouse serum. Polyclonal antimouse γ-chain antiserum was made in goats by injecting a mixture of papain Fc fragments from MOPC-31C, Adj-PCS, and MOPC-195 myeloma proteins, which correspond to the γG1, γG2a, and γG2b subclasses of γG. Antimouse γG3 was made in rabbits with the J-606 (kindly supplied by Dr. Howard Grey) and absorbed with a mixture of purified MOPC-31C, Adj-PCS, and MOPC-195 myeloma proteins. Multiple controls for the monospecificity of each antiserum were done, including immunofluorescent staining of myeloma tumors representative of the major immunoglobulin classes. All sera injected into newborn mice were submitted to the following treatment. The γ-globulin fraction was obtained by sequential precipitation with

3 Mattioli, C. A., and T. B. Tomasi, Jr. 1973. The use of immunoprecipitated antigen for the preparation of antisera. Manuscript in preparation.
18%, 12%, and a second 12% Na₂SO₄. After exhaustive dialysis of the precipitate against saline and clarification by ultracentrifugation, the protein concentration was adjusted to 100 mg/ml and the antisera sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.).

For the Jerne hemolytic plaque assay, facilitating antisera monospecific for mouse α-chains were made in rabbits using the MPC-1 myeloma protein. The previously described polyvalent goat antimmune γ-chain antisera was used to facilitate γG PFC. The optimal dilution of the anti-γA-antiserum was 1 to 20 and of the anti-γG-antiserum was 1 to 100.

Histological Studies.—The immunofluorescent technique of Coons (29), with the modifications previously described, was used to enumerate plasma cells belonging to different immunoglobulin classes.

The plasma cell population of the intestine was evaluated in sections from the initial portion of the small bowel by counting the number of γA-cells in 100 consecutive longitudinally cut villi. Since very few γM- and γG-plasma cells are found in the normal mouse intestine, quantitation of these plasma cell classes was not attempted.

In the case of the spleen, one part of the organ representing ½ to ¾ of the total spleen weight was sectioned and used for histological studies. The remaining portion of the spleen was used for the Jerne plaque assay. The figures reported in the results section refer to counts of γM-, γA-, and γG-plasma cells in two sections, cut 12 μm apart in the tissue block to include different cellular groups.

RESULTS

Suppression of Antibody Synthesis in Mice Treated with Antiglobulins.—Experiments were first attempted in which animals were injected with approximately 10 mg of globulin in 0.1 ml at 24-h intervals beginning within 12 h of birth. However, this regimen resulted in a high incidence of deaths in the first few days of life. These deaths occurred randomly among controls, anti-β₅-, and anti-α-treated mice and were attributed to the trauma of constant handling and injections. No early deaths occurred when animals were similarly injected at 48-h intervals; therefore, this schedule was maintained throughout these experiments.

The duration of antiglobulin treatment necessary for effective immunosuppression in vivo was not known. Therefore, experiments were done in which antiglobulin treatment was continued from birth through young adulthood to within 48 h of sacrifice for antibody assays. Antibody responsiveness to SRBC was assessed by measuring PFC and humoral hemagglutinating antibody (serum HA) titers 4 and 10 days after primary intraperitoneal injection of 5 × 10⁶ SRBC. The effects of continuous antiglobulin treatment on the antibody response to SRBC are shown in Table I, exps. 1 (day 4 assays) and 2 (day 10 assays). In exp. 1, anti-γA treatment somewhat depressed direct PFC numbers, but showed no effect on 2-ME-sensitive serum antibody levels suggesting little effect on γM-antibody. Anti-γM-treated mice had no detectable humoral or cellular antibody response. Day 10 assays (exp. 2) show that mice treated with anti-γA have partially depressed γG PFC responses and essentially complete suppression of γA PFC. Again, as in the first experiment, there is no

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significant effect on 2-ME-sensitive serum antibody, although 2-ME-resistant antibody is lowered. Anti-γM-treated mice are completely suppressed in all immunoglobulin classes.

In a second series of experiments, antiglobulin treatment was discontinued 6 days before injection of antigen. Day 4 antibody assays (Table I, exp. 3) show that anti-γA treatment has no suppressive effect on either direct PFC or serum HA levels, while the antibody responses of anti-γM-treated mice were significantly suppressed. Antiglobulin treatment in these animals was terminated 10 days before time of assay. Day 10 antibody assays (exp. 4) that were performed 16 days after the last injection of antiglobulins demonstrated that even

| Table I |

| Exp. | Treatment | No. of animals | Duration of treatment | Total done | Time given | Mean no. PFC/10⁶ spleen cells ± SE | Mean reciprocals of anti-SRBC HA titers ± SE |
|------|-----------|----------------|----------------------|------------|------------|--------------------------------|--|
| 1    | Controls  | 6              | 0-21 100 20 24      | 130 ± 17   | 3.3 ± 0.3 | 0                         |
|      | Anti-γA   | 3              | 0-21 100 20 24      | 64 ± 9     | 3.7 ± 0.3 | 0                         |
|      | Anti-γM   | 12             | 0-21 100 20 24      | 0          | 0          | 0                         |
| 2    | Controls  | 4              | 0-27 120 20 30      | 77 ± 9     | 4.0 ± 0.2 | 2.8 ± 0.3 |
|      | Anti-γA   | 6              | 0-27 120 20 30      | 50 ± 10    | 3.3 ± 0.7 | 1.3 ± 0.3 |
|      | Anti-γM   | 5              | 0-27 120 20 30      | 0.6 ± 0.4  | 1.6 ± 0.3 | 0                         |
| 3    | Controls  | 13             | 0-26 80 20 36       | 236 ± 40   | 5.8 ± 0.5 | 0                         |
|      | Anti-γA   | 8              | 0-26 80 20 36       | 402 ± 142  | 5.3 ± 0.5 | 0                         |
|      | Anti-γM   | 7              | 0-26 80 20 36       | 135 ± 36   | 1.6 ± 0.3 | 0                         |
| 4    | Controls  | 2              | 0-26 80 20 32       | 194 ± 4   | 3.6 ± 0.2 | 0                         |
|      | Anti-γA   | 5              | 0-26 80 20 32       | 110 ± 8   | 5.6 ± 0.5 | 0                         |
|      | Anti-γM   | 6              | 0-26 80 20 32       | 22 ± 21   | 0.7 ± 0.7 | 0.5 ± 0.5 |

with treatment of shorter duration, anti-γM-globulin retained its potent immunosuppressive activity. Anti-γA also suppressed, but only the γA PFC response, with little effect on the other antibody classes. The greater numbers of PFC in control animals from exps. 3 and 4 compared with those from exps. 1 and 2 can be attributed to age differences at the time of antigenic stimulation.

It was considered of practical as well as theoretical importance to determine the necessary duration of treatment and the concentration of anti-γM required for immunosuppression. The results presented in Table II show that mice in which anti-γM treatment was terminated 2 days after birth were suppressed to almost the same extent as mice that received continuous long-term treatment through young adulthood. Mice that received various concentrations of anti-γM by the same injection regimen showed marked suppression of PFC antibody
It is interesting that from the data obtained at the lowest dilution that γM recovery may precede that of γG and γA. Also to be noted is that 20 mg of anti-γM elicited similar degrees of immunosuppression when injected on a short-term regimen in concentrated form (Table II) or on a long-term regimen in diluted form (Table III).

**Suppression of Serum Immunoglobulin Levels in Mice Treated with Anti-globulins.**—Serum immunoglobulin levels in antiglobulin-treated mice are pre-

| Exp. no. | Treatment | Treatment | No. of animals | Duration of treatment | Total mg | Time SRBC given | Time of PFC assay | Mean no. PFC/10^6 spleen cells ± SE |
|----------|-----------|-----------|----------------|----------------------|----------|----------------|------------------|-----------------------------------|
| 1        | Controls  | Anti-γM   | 3              | 0-21                 | 100      | 20             | 24               | 128 ± 23                          |
|          | Anti-γM   |           | 3              | 0-21                 | 100      | 20             | 24               | 0                                 |
|          | Anti-γM   |           | 3              | 0-2                  | 20       | 20             | 24               | 13 ± 8                            |
| 2        | Controls  | Anti-γM   | 4              | 0-27                 | 120      | 20             | 30               | 77 ± 9                            |
|          | Anti-γM   |           | 5              | 0-27                 | 120      | 20             | 30               | 0.6 ± 0.4                         |
|          | Anti-γM   |           | 8              | 0-2                  | 20       | 20             | 30               | 11 ± 2.4                          |

**TABLE III**

Effect of Duration of Treatment with Anti-γM on the Primary Plaque-Forming Cell Response

| Treatment | No. of animals | Duration of treatment | Total mg | Time SRBC given | Time of PFC assay | Mean no. PFC/10^6 spleen cells ± SE |
|-----------|----------------|----------------------|----------|----------------|------------------|-----------------------------------|
| Controls  | 3              | 0-26                 | 80       | 32             | 42               | 104 ± 11                         |
| Anti-γM   | 6              | 0-26                 | 80       | 32             | 42               | 22 ± 21                          |
| Anti-γM   | 2              | 0-26                 | 40       | 32             | 42               | 19 ± 2                          |
| Anti-γM   | 3              | 0-26                 | 20       | 32             | 42               | 55 ± 24                          |

The results clearly demonstrate a selective deficiency of serum γA- immunoglobulins in anti-γA-treated mice. Serum-γG3 concentrations were found to be normal in anti-γA-treated animals. A selective suppression of γA occurred concomitantly in the serum (mean γA level = 1) and intestinal washings (mean γA level = 1.25) of four 40-day-old animals treated from birth with 180 mg of anti-γA. Four control animals similarly treated with normal goat globulins had a mean serum-γA level of 7 and intestinal fluid-γA level of 4 (see footnote of Table IV for method of measurement). Rabbit antibodies to mouse-γA were detectable by double diffusion analysis in the sera of only 2 of the 16 animals treated with anti-γA-globulin. Immunoglobulin levels
of all classes including γG3 were suppressed in animals treated with anti-γM-globulin. Circulating antibody to mouse-γM was below the level of detection by double diffusion analysis in all μ-treated mice. As shown by exp. 4, Table IV, antiglobulin-induced suppression of serum immunoglobulin levels was observed for more than 2 wk after termination of treatment, which again suggests this type of inhibition has long-lasting effects.

**Immuno/fluorescent Analysis of Lymphoid Tissues.**—Tissue sections of spleen and intestine from 30-day old mice given long-term treatment with anti-γM-, anti-γA-, or normal γ-globulins were examined for plasma cells containing γM, γA, and γG by the indirect immunofluorescent technique. Due to the relatively small numbers of plasma cells normally present in the spleen of animals of this age two sections were scanned. These sections were cut 12 μm apart in the tissue block to include different cell clusters and to compensate for the irregular distribution of plasma cells. As depicted in Fig. 1 (3M, 3A, 3G) and quantitated in Table V, the spleens of anti-γM-treated animals are completely devoid of all immunoglobulin-containing cells. Compared with controls, anti-γA-treated animals showed no differences in the numbers of γM- and γG-containing plasma cells in their spleens, while γA-plasma cells were substantially diminished (Fig. 1 [2M, 2A, 2G] and Table V). Thus, the effect of anti-γA treatment on splenic plasma cell development was concordant with its effect on antibody and immunoglobulin synthesis.

**TABLE IV**

| Exp. no. | Treatment | No. of animals | Duration of treatment | Total dose | Time SRBC given | Time of serum collection | Mean serum immunoglobulin levels* ± SE |
|----------|-----------|----------------|----------------------|------------|------------------|---------------------|--------------------------------------|
| 1        | Controls  | 6              | 0-21                 | 100        | 20               | 24                  | γM: 5 ± 0, γA: 4 ± 0, γG: 85 ± 24, γG2: 5 ± 1 |
|          | Anti-γM   | 6              | 0-21                 | 100        | 20               | 24                  | 8 ± 0, 7 ± 1, 0, 17 ± 5, 2 ± 1     |
| 2        | Controls  | 7              | 0-27                 | 120        | 20               | 30                  | γM: 3 ± 0.4, γA: 99 ± 5, γG: 32 ± 9 |
|          | Anti-γA   | 6              | 0-27                 | 120        | 20               | 30                  | 8 ± 0, 0, 53 ± 7, 17 ± 3           |
|          | Anti-γM   | 12             | 0-27                 | 120        | 20               | 20                  | 1 ± 1, 0, 19 ± 6, 9 ± 5            |
| 3        | Controls  | 13             | 0-26                 | 80         | 32               | 36                  | γM: 10 ± 1, 12 ± 0.3, 28 ± 6, 13 ± 3 |
|          | Anti-γA   | 4              | 0-26                 | 80         | 32               | 36                  | 16 ± 0.4, 30 ± 25, 21 ± 5          |
|          | Anti-γM   | 4              | 0-26                 | 80         | 32               | 26                  | 6 ± 2, 0, 16 ± 6, 6 ± 1            |
| 4        | Controls  | 4              | 0-26                 | 80         | 32               | 42                  | γM: 12 ± 4, 12 ± 0.5, 48 ± 16, 12 ± 4 |
|          | Anti-γA   | 3              | 0-26                 | 80         | 32               | 42                  | 13 ± 3, 0, 37 ± 14, 11 ± 3         |
|          | Anti-γM   | 3              | 0-26                 | 80         | 32               | 42                  | 7 ± 3, 0, 19 ± 7, 7 ± 1            |

*Expressed as the highest reciprocal serum dilution showing distinct precipitin bands in Ouchterlony gel diffusion using commercial (Meloy) class-specific antisera.
Fig. 1. Immunofluorescence of spleen sections from control, anti-γA-, and anti-γM-treated mice. Tissue sections were stained with rabbit antimouse γM(M), γA(A), and γG(G). IM, IA, and IG show spleen sections from mice treated with normal γ-globulin. 2M, 2A, and 2G are spleen sections from anti-γA-treated mice and demonstrate a selective absence of γA-containing cells. Sections 3M, 3A, and 3G are from anti-γM-treated mice and exhibit the virtual complete elimination of immunoglobulin-containing cells. × 184.
a complete absence of the γA-plasma cells that are normally present in control animals (Fig. 2 A and B and Table V). Surprisingly, the numbers of γA-plasma cells in the lamina propria of anti-γA-treated animals did not differ significantly from controls (Fig. 2 C and Table V). Linear deposits of rabbit γ-globulin in the lamina propria and along the basement membrane were observed in these animals (Fig. 2 D), indicating that the anti-γA gained access to the lamina propria. However, a quantitation of the relative concentration of the

| Treatment | γM-plasma cells | γA-plasma cells | γG-plasma cells | No. of γA-plasma cells/100 villi | γA | γM |
|-----------|----------------|----------------|----------------|---------------------------------|-----|-----|
| Control   | 83 ± 110       | 2 ± 4          | 16 ± 9         | 15 ± 20                         | 61 ± 30 | 1,329 ± 1,179 ± 67 |
| Control   | 54 ± 129       | 11 ± 0         | 21 ± 3         | 1,061                          |
| Control   | 103 ± 128      | 4 ± 0          | 34 ± 13        | 1,053                          |
| Control   | 88 ± 133       | 76 ± 23        | 104 ± 257      | 1,294                          |
| Anti-γA   | 95 ± 48        | 2 ± 0          | 179 ± 107      | 123                            |
| Anti-γA   | 118 ± 12       | 0 ± 0          | 30 ± 32        | 1,060                          |
| Anti-γA   | 15 ± 18        | 0 ± 0          | 30 ± 39        | 955                            |
| Anti-γA   | 89 ± 113       | 8 ± 8          | 72 ± 69        | 896                            |
| Anti-γA   | 133 ± 151      | 9 ± 12         | 103 ± 76       | 1,374                          |
| Anti-γA   | 62 ± 91        | 4 ± 4          | 102 ± 87       | 605                            |
| Anti-γM   | 0 ± 0          | 0 ± 0          | 0 ± 0          | ND                             |
| Anti-γM   | 0 ± 0          | 0 ± 0          | 0 ± 0          | 0                              |
| Anti-γM   | 0 ± 0          | 0 ± 0          | 0 ± 0          | 0                              |

* Animals were treated from birth through day 27 with 120 mg of gamma globulins and were 30 days old at the time of sacrifice.
† Plasma cell counts from two different spleen sections cut 12 μm apart.
§ Mean plasma cell numbers ± standard error.

anti-γA-globulins in the circulation vs. lamina propria interstitial fluid was not possible.

**Histology of Anti-γA- and Anti-γM-Treated Mice.**—Thymus, spleen, and intestine from anti-γA-treated mice showed a normal microscopic architecture and did not differ from control animals when stained with hematoxylin-eosin.

**Fig. 2. Immunofluorescence of sections of intestine stained for γA-containing cells.** (A), intestine from control mouse showing significant numbers of lamina propria γA-plasma cells; (B), intestine from anti-γM-treated mouse with lamina propria completely devoid of fluorescent cells; (C), intestine from anti-γA-treated mouse showing abundance of γA-plasma cells in numbers approximately equivalent to control; (D), intestine from anti-γA-treated mouse stained with fluorescein-conjugated goat antirabbit γ-globulin demonstrating deposition of rabbit γ-globulin within the lamina propria. × 211.
and methyl green-pyronin. Specifically, there was no indication of mucosal inflammation of the gastrointestinal tract, nor villus changes compatible with malabsorption syndrome.

In the anti-μ-treated mice, the gross appearance of the thymus and intestine was normal, while the spleen was reduced in size, markedly so in the runted animals. This was clearly reflected in the low cell counts of spleen cell suspensions from μ-treated animals (Table VI). Histologically, the thymus had a normal cortex and medulla. Hassall's corpuscles, lymphocytes, and epithelial cells were unremarkable. The spleen had primary follicles, but a marked reduc-

**TABLE VI**

Spleen Cell Counts of Antiglobulin-Treated Mice

| Exp. no. | Treatment | No. of animals | Age | Mean cell count (X 10⁶) ± SE |
|----------|-----------|----------------|-----|----------------------------|
| 1        | Controls  | 3              | 30  | 212 ± 26                   |
|          | Anti-γA   | 3              | 30  | 245 ± 21                   |
|          | Anti-γM*  | 3              | 30  | 104 ± 39                   |
| 2        | Controls  | 3              | 24  | 168 ± 31                   |
|          | Anti-γA   | —              | 24  | —                          |
|          | Anti-γM*  | 6              | 24  | 83 ± 5                     |
| 3        | Controls  | 4              | 42  | 137 ± 13                   |
|          | Anti-γA   | 6              | 42  | 155 ± 12                   |
|          | Anti-γM*  | 3              | 42  | 91 ± 36                    |
| 4        | Controls  | 3              | 36  | 165 ± 18                   |
|          | Anti-γA   | —              | 36  | —                          |
|          | Anti-γM*  | 8              | 36  | 94 ± 10                    |

* These data do not include severely runted animals who rarely survived longer than 15 days.

**Evidence for Runting Syndrome in Anti-γM-Treated Mice.**—A high frequency of runting (See Fig. 3) in anti-γM-treated mice was observed in preliminary
experiments designed to determine the amount and frequency of antiglobulin injections that newborn mice could sustain and remain healthy. Runting occurred in all of 30 animals from six litters injected at 24-h intervals starting within 12 h of birth with 20 mg of anti-\( \gamma \)-globulins in 0.2 ml. 30 control littermates injected with saline grew and developed normally. Severe runting was first observed by 4 days at which time the injections were stopped. In this experiment the treated mice that received approximately 80 mg of anti-\( \gamma \)-globulins in four injections during the first 4 days of life all remained runted until their death, which occurred in all runted animals within 15 days. Normal growth and development was observed in all animals treated with two anti-

\[ \gamma A \]-globulin preparations, two anti-\( \gamma G \) preparations (unpublished observations), and several pools of control normal rabbit or goat \( \gamma \)-globulins. Runting was consistently observed in mice treated with three qualitatively different anti-\( \gamma \)-globulin preparations at the same total protein concentrations. The establishment of this condition is dependent upon the dose of anti-\( \gamma \)-globulin injected since a series of 20-mg injections induced 100% lethal runting, 10-mg injections induced 20% runting, while 5- or 2.5-mg injections of anti-\( \gamma \)-globulin produced immunosuppression but no severe runts. While severely runted animals rarely lived beyond 15 days, those anti-\( \gamma \)-treated littermates that escaped this condition survived as long as controls or anti-\( \gamma A \)-treated animals. Illustrated in Table VII, however, are the lower weights of the surviving anti-\( \gamma \)-treated animals compared with controls and anti-\( \gamma A \)-treated animals, which suggests a gradation of wasting extending from short-lived severe runts, to partial runts that survive, to animals approaching normal weight and develop-

Fig. 3. 10-day old antiglobulin-treated littermates. The severely runted animal received 40 mg of rabbit antimume \( \gamma \)-globulin in four 10-mg intraperitoneal injections during the first 4 days of life. The control littermate was similarly treated with an equivalent amount of normal rabbit \( \gamma \)-globulin. At day 10 the control animal weighed 5.2 g and the runted animal weighed 2.7 g.
ment. Runtling could be initiated by a single 10 mg injection only if it was administered within 12 h of birth. Further attempts to induce runts with various doses and numbers of injections in mice older than 1 day were unsuccessful. In addition, after the 1st day, continued injections did not increase the number of runts but did advance the time of death of those already runted.

**DISCUSSION**

This study demonstrates that treatment of conventional newborn mice with anti-\(\gamma\)M-globulin causes extensive immunosuppression that affects the response in all immunoglobulin classes. Similar observations have recently been made by others both in vitro (24) and in vivo using germfree (21) as well as conventional (22) animals. Several alternative explanations for the immunosuppressive activity of anti-\(\gamma\)M on \(\gamma\)G and \(\gamma\)A in addition to \(\gamma\)M synthesis have been put forth involving either the sequential and/or simultaneous expression of \(\gamma\)M-, \(\gamma\)G-, and \(\gamma\)A-antigen receptors on virgin precursor lymphocytes (21, 22, 24). Some evidence has accumulated that suggests that antoglobulins directly suppress B lymphocytes based on: (a) in vitro reconstitution experiments with partially purified macrophages, B, and T cell preparations showing suppression of the immune response when B cells were exposed to anti-\(\gamma\)M before culture,
but not when only macrophages and/or T cells were similarly exposed (24); (b) studies showing suppression by anti-γM of antibody responses to thymus-independent antigens (21, 22) as well as suppression in genetically athymic mice (22); and (c) demonstration of normal morphological development in thymuses and "thymus-dependent" areas of lymphoid tissues in anti-γM-suppressed animals (21). It is apparent from homograft studies (30) that the T lymphocyte population that mediates transplantation immunity is not affected by anti-γM. In addition, the collaborating T lymphocyte is not suppressed by exposure to anti-γM before in vitro (24) or in vivo (22) reconstitution.

Although the exact cellular basis for immunosuppression by anti-γM remains speculative, presumably the interaction of anti-γM with cell surface antigen receptors of the μ-chain type is a prerequisite. We presently favor the concept that the generalized suppression induced by anti-γM depends on the initial presence of a population of multipotential B lymphocytes with surface γM-receptors from which all the various classes of plasma cell clones arise through a maturation process probably occurring initially within the microenvironment of the mammalian bursa equivalent (BE). The severe runting that accompanies the early administration of high concentrations of anti-γM shown in this study could, therefore, be a manifestation of a severely crippled immune system that is completely devoid of mature B lymphocytes although just how this would affect growth and development is at present unclear. That runting was not observed in previous studies (21, 22), probably results from the smaller effective doses of anti-γM employed.

Although this concept, as well as much of the previous work cited by others, emphasizes the B lymphocytes as the site of action for antiglobulin, the possibility that a subpopulation of T lymphocytes is involved should be considered. Thus, runting as well as the generalized immunosuppression induced by anti-γM could be related to the inactivation of a T lymphocyte population expressing γM-antigen receptors. Some evidence for surface γM on T cells has been previously reported (20). The observations that cell-mediated immunity is intact in these animals and that the collaborating "helper" T lymphocytes necessary for the induction of certain antibody responses are unaffected by anti-γM does not negate this premise since there is evidence that there may be several functional classes of T lymphocytes (31). Anti-γM could block the function of an ontogenetic regulator T lymphocyte population that is distinct from the helper cell and is necessary to induce further differentiation of multipotential precursor B lymphocytes toward γM-, γG-, and γA-plasma cells.

The thymus appears to be of normal size in runted animals in contrast to the profound atrophy of this lymphoid organ commonly observed in other forms of wasting disease. However, this does not preclude an anti-γM-induced thymic defect of a more subtle nature. Because of the immediate arrest in development that is noted within 4 days in anti-γM-runted animals, it is not unreasonable to
suspect that endocrine alterations are affecting ontogenetic maturation. In this regard, a type of runting syndrome, perhaps involving the thymus, has been reported (32, 33) after adrenalcorticosteroid administration. Furthermore, it has been demonstrated that adrenalectomy ameliorates one form of wasting disease (34). In spite of certain common features that exist between the wasting diseases induced by cortisone and anti-γM, the normal gross and histological findings in the thymus of anti-γM-induced runts are not consistent with the profound atrophy of this lymphoid organ observed after the administration of cortisone. Therefore, at present the precipitating etiological factors involved in antiglobulin-induced runting remain highly speculative and experiments are currently in progress to explore further the pathogenesis of this syndrome and its relationship with drug-induced wasting (33) as well as other forms of wasting disease (35–38).

The possibility should be considered that antiglobulin-induced suppression is analogous to chronic allotype suppression in mice (15) that may have as its cellular basis an active suppressing process that is transferable by spleen cells but not serum from suppressed animals. Jacobson et al. (16) suggest that there is a population of cells in the spleen that arises as a consequence of exposure to antiglobulin, which is capable of recognizing potential immunoglobulin-producing cells and preventing their expression. Their preliminary evidence that the anti-allotype-induced suppressor is a thymus-derived cell suggests that T lymphocytes may play an important regulatory role on B lymphocytes and their immunoglobulin products. The finding that prolonged incubation of anti-γM with spleen cells is necessary to obtain antiglobulin-induced suppression of antibody synthesis in vitro (24) also suggests a suppression mechanism more complex than saturation of receptors and prevention of antigenic stimulation.

Clones of cells with the capacity to synthesize γG are thought to arise by conversion from B lymphocytes expressing γM-receptors. For a few generations, cells that have newly developed γG-receptors may also express γM-receptors (γM-γG "double producers"). As postulated by Lawton et al. (21), γA-producing cells may arise from γG-cells by a similar sequence of ontogenetic events. Treatment with anti-γG should, therefore, suppress γG, and if most or all γA-cells arise from γG-precursors, a significant suppressive effect by anti-γG on γA should be observed. Our preliminary experiments designed to investigate this have been unsuccessful and thus far, our results with anti-γG treatment have been equivocal except that these antiglobulins are not able to induce runting. Suppression by anti-γG has been reported in vivo (22) and in vitro (24) but the effects are partial. The reason for partial and not complete suppression by anti-γG in vitro is not apparent. However, in vivo, perhaps the large amounts of maternal γG present in the circulation of newborn animals eliminates much of the anti-γG before it reaches the immune mechanism. The presence of circulating maternal γG-globulins may also account for the inability of anti-γM treatment to completely eliminate serum-γG1 and γG2 as observed in this study.
A similar persistence of these two serum immunoglobulins in anti-γM-treated animals was noted by others (21, 22) and was attributed to the presence of maternally derived γG1- and γG2-antibodies (22). Our data showing that splenic γG PFC are suppressed (Table I) in anti-γM-treated animals that have significant levels of serum-γG (Table IV) would seem to be in agreement with this reasoning. However, it is also possible that some of the nonsuppressible residual serum-γG derives from γG-producing cell lines that were already fully differentiated and peripheralized before the start of antiglobulin treatment.

Assuming that the γA-plasma cell precursor is a lineage arising from γG-cells, treatment with anti-γA should suppress only γA with possibly some slight effect on γG via γG-γA-clones, and should exert no effect on γM. Our data, as well as in vitro (24) experiments, are consistent with this prediction. However, our findings do not exclude the possibility that some γA-cells may arise directly from γM-cells without an intermediate γG stage. In fact, if γM-precursors differentiate into γA-cells within the gastrointestinal tract, as suggested by this study (see below), the paucity of γG-cells in the lamina propria is most easily explained by a direct γM-γA conversion.

While animals treated with anti-γA had selectively suppressed serum-γA, as well as splenic γA-plasma cells, the γA-plasma cells in the lamina propria of the gastrointestinal tract were present in numbers approximately equivalent to those in the controls. The reason for this rather striking dissociation is not clear. Complete inaccessibility of the antiglobulin to intestinal plasma cells is not involved since linear deposits of heterologous globulin derived from the administered antisera were seen within the lamina propria of most anti-γA-treated animals. Also, the possibility of qualitative differences between the γA-receptors on intestinal γA-plasma cells vs. splenic γA-plasma cells seems unlikely since antisera directed against both secretory and nonsecretory types of γA were used in these experiments with similar results. Moreover, no differences between the α-chains of serum and secretory γA have been observed in studies to date (39). A speculative suggestion that is consistent with the observed dissociation between systemic and secretory γA-cells after anti-γA treatment and the complete suppression of all γA-cells by anti-γM is as follows: As mentioned previously, γA-cells may arise from precursor lymphocytes having surface γM possibly via an intermediate γG stage. These early maturation events probably occur in the mammalian BE, perhaps the bone marrow itself. In any case, anti-γM-globulin has access to the γM-precursor cells either in the BE or circulation in amounts sufficient to inactivate them before peripheralization. On the other hand, in the anti-γA-treated animals the bone marrow-derived μ-chain bearing precursor cells are not affected and normally seed the intestinal lamina propria directly or perhaps via Peyer’s patches as suggested by recent cell migration studies (40). We postulate that differences in the concentration of the antiglobulin reagent (anti-γA) in the circulation and spleen vs. the gastrointestinal lamina propria and Peyer’s patches allows differentiation into γA-cells to occur.
in the gut but not in peripheral lymphoid tissues. Important in this regard is the observation that antiglobulin-induced suppression is reversible and not the result of a cytotoxic action (22, 24). Alternatively, some evidence exists (41) that most or all of the γA-cells normally found in peripheral lymphoid tissues such as the spleen originate in the gut, and while the concentration of anti-γA in the lamina propria may be insufficient to prevent differentiation, it does inhibit cell migration. An additional factor could be the larger antigenic stimulation to the secretory cells in these conventionally fed animals. Effective suppression by anti-γA may also be hindered by continuous recruitment from preceding classes of plasma cell precursors via the switch mechanism. In fact, conversion from the preceding precursor may actually be enhanced by depletion of the class of precursor that follows in the ontogenetic sequence.

Convincing evidence that the rather striking serum-γA deficiency observed in this study is not due solely to peripheral blocking of endogenously synthesized γA by excess antimouse γA comes from the experiments showing that (a) anti-γA treatment selectively suppresses splenic γA PFC; (b) immunofluorescent analysis of splenic immunoglobulin-containing plasma cells from anti-γA-treated animals shows a selective deficit of γA-containing cells, and (c) the majority of animals have no demonstrable circulating anti-γA at the time of testing. Also Manning (42) states that a peripheral block is unlikely as the sole explanation since he found no suppression of serum or fecal γA when anti-γA treatment was begun on day 24, whereas a transient γA deficiency ensued if treatment was initiated at birth.

Evidence has been presented both in dogs (43) and mice (44, 45) that serum-γA may be derived in large part from synthesis in cells located in the gastrointestinal tract. The marked suppression of serum-γA with essentially normal numbers of γA-cells in the gut lamina propria observed in anti-γA-treated animals is at first glance contrary to this hypothesis and would be analogous to the patients that have been reported showing a similar dissociation between serum and secretory γA (46). However, much of the γA normally added to the serum by the lamina propria cells could be prevented from reaching the circulation by the anti-γA present in the lamina propria and/or circulating anti-γA could remove γA as rapidly as it is added leaving the concentration of serum-γA reduced below the level of detectability by the relatively insensitive methods employed in this study. This seems reasonable particularly since the concentration of γA in normal adult mouse serum is small, of the order of 1/10 that of the human. Another possibility is that the anti-γA present in the lamina propria inhibits the cellular release of γA. In the latter case the plasma cells would contain but not secrete γA, a situation which has been reported in some cases of human myelomas (47).

A final point that bears mentioning is that on the basis of the model for sequential plasma cell development that proceeds from γM to γG to γA, it would be predicted that the order of recovery from anti-γM-induced suppres-
sion would also progress from $\gamma M$ to $\gamma G$ to $\gamma A$. In agreement with this proposed sequence of events, our experiments indicate that $\gamma M$ is the first immunoglobulin to begin to recover after termination of treatment. This observation is in contrast to that of Manning and Jutila (22) who showed that during prolonged anti-$\gamma M$ treatment, serum-$\gamma M$ remains suppressed, $\gamma G$ begins to recover slightly, and $\gamma A$ levels often return to normal values. The reason for this difference in results is not apparent.

**SUMMARY**

Conventional Swiss mice were treated from birth with intraperitoneal injections of anti-immunoglobulins in an attempt to create an experimental dysgammaglobulinemia. Mice treated with anti-$\gamma M$ were immunologically suppressed in all immunoglobulin classes as determined by serum antibody titers, splenic plaque-forming cells, serum immunoglobulin levels, and immunofluorescent analysis of plasma cells in lymphoid tissues. Treatment immediately after birth with high concentrations of anti-$\gamma M$ leads to a developmental arrest characterized by severe immunosuppression, failure to gain weight, and premature death. The pathogenesis of anti-$\gamma M$ runting syndrome is unknown. Animals similarly treated with anti-$\gamma G$, anti-$\gamma A$, or control normal goat or rabbit $\gamma$-globulins developed normally. The frequency of occurrence and severity of anti-$\gamma M$-induced runting syndrome is dependent upon the concentration of anti-$\gamma M$-globulin administered.

Administration of anti-$\gamma A$ resulted in a selective $\gamma A$ deficiency that was characterized by a marked reduction in serum-$\gamma A$ and an absence of $\gamma A$-containing cells in the spleen. However, essentially normal numbers of plasma cells were found in the gastrointestinal lamina propria of anti-$\gamma A$-treated animals concomitantly with suppressed levels of $\gamma A$ in intestinal fluids.

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