

**SUPPRESSION OF REAGINIC ANTIBODY (IgE) FORMATION  
IN MICE BY TREATMENT WITH ANTI- $\mu$  ANTISERUM\***

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Neonatally initiated injection of anti- $\mu$  antibodies has previously been shown to suppress the formation of IgM, IgG1, and IgG2 in both conventionally reared (1, 2) and germ-free (3) mice. Production of IgA can also be blocked by anti- $\mu$  antibodies so administered (2-5), but IgA suppression requires somewhat higher dosages of suppressive antibody and is a rather unstable condition tending toward recovery (4, 6). Similar panspecific immunosuppressive effects of anti- $\mu$  antibodies have been observed in mouse cell cultures as well as in cell culture and in vivo systems for several other species (reviewed in 7).

Lack of an appropriate test system has delayed a definitive answer to the question of reaginic antibody (IgE) suppression with anti- $\mu$  antibodies. From their in vitro studies of rabbit immunocytes, Kishimoto and Ishizaka (8) concluded that anti- $\mu$  antibodies cannot suppress secondary IgE responses in this species, but they were unable to examine primary IgE responses in cell culture. In a study employing a relatively light anti- $\mu$  suppressive regimen in neonatal mice, Dwyer et al. (9) did not achieve suppression of IgE responses toward subsequent antigenation with ovalbumin in alum, suggesting to them an ontogenetic development for IgE-bearing cells independent of that leading to IgM, IgG, or IgA production. Our own early attempts at suppression of murine IgE responses to ovalbumin in alum using more massive regimens of neonatal anti- $\mu$  treatment were frustrated by the severe depression (or masking) of IgE production which large doses of normal rabbit serum (NRS) frequently produce in control mice. Further, although administration of rabbit serum itself can be shown to generate a reaginic response in mice, both the response and its suppression have proven to be quite variable and require careful interpretation (Manning, J. K., N. D. Reed, and D. D. Manning; to be published). Utilizing the reaginic response of mice to an infection with an intestinal nematode, *Nippostrongylus brasiliensis*, we now present data that demonstrate active suppression of IgE responses by anti- $\mu$  antibodies, suggesting that IgE-producing cells, like those of all other immunoglobulin classes examined to date, arise from IgM-bearing precursoral cells.

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## Materials and Methods

**Anti- $\mu$  Antiserum.** Preparation of the anti- $\mu$  antiserum has been detailed previously (10). Briefly, a rabbit was immunized repeatedly with highly purified IgM (MOPC 104E protein) emulsified in Freund's adjuvants, and the resultant antiserum was rendered  $\mu$ -specific by column absorption with other immunoglobulins and serum components conjugated to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). Monospecificity was verified by Ouchterlony gel diffusion against purified IgM, IgG1, IgG2, and IgA as well as immunoelectrophoresis against whole mouse serum. This antiserum at a dilution of 1:256 formed a distinct precipitin band when diffused in gel against IgM (0.33 A<sub>280 nm</sub> U/ml). Both the antiserum and the control NRS were absorbed twice with 3% mouse erythrocytes and filter sterilized before use.

**Mice and Suppression Protocol.** BALB/c mice were used throughout the study. Within 14 h of birth, each of three litters was divided into three groups. One group in each litter was injected intraperitoneally with 0.05 ml of anti- $\mu$  serum, the other two receiving an identical injection of NRS or phosphate-buffered saline (PBS); further injections of 0.05, 0.07, 0.07, 0.08, and 0.08 ml were made on days 2, 4, 6, 8, and 10, respectively. Thereafter all mice received a 0.10 ml injection of PBS or diluted NRS or anti- $\mu$  serum (both diluted 1:2.5 in PBS) every Monday, Wednesday, and Friday until termination of the experiment.

**Worm Infection.** At approximately 40 days of age all mice received a subcutaneous injection of 300 third-stage (infectious) larvae of a mouse-adapted strain of *N. brasiliensis* (11). 2 wk later all mice were reinfected with 300 such larvae and 13 days thereafter were exsanguinated retro-orbitally.

**Immunological Determinations.** Serum IgM levels were determined by the serial dilution Ouchterlony assay of Arnason et al. (12); thus a given IgM level was designated as the reciprocal of the highest twofold serum dilution producing a distinct precipitin line against a commercial (Meloy Laboratories Inc., Springfield, Va.) anti- $\mu$  serum of standardized activity. A similar assay using NRS diluted 1:4 as antigen was used to quantitate anti-NRS precipitin responses in the test mice. Examination of all anti- $\mu$ -treated mice for circulating anti- $\mu$  antibodies was made by an assay identical to that used to quantitate the antiserum injected. In all of these assays, a value of zero was assigned to any serum showing no reaction when tested undiluted.

Reaginic antibody responses were assayed with the passive cutaneous anaphylaxis (PCA) test performed in rats (13, 14). For each serum, 0.10-ml aliquots of a series of twofold dilutions were injected intradermally into a Sprague Dawley test rat. Each injection series was begun with undiluted serum with two exceptions, for which serum quantity necessitated a starting point of 1:4. 24 h later the test rats received an intravenous injection of saline containing 2,000 worm equivalents of soluble *N. brasiliensis* antigen and 5 mg Evans Blue dye. The *Nippostrongylus* antigen was prepared as described by Ogilvie (15). A positive PCA reaction was recorded for any injection site showing a 3 mm or greater diameter of skin bluing 30 min after challenge. As with the serum assays, a value of zero was assigned to any test in which no reaction was detected using undiluted serum. Spot checks were also made for loss of serum PCA activity after heating at 56°C for 90 min whenever test serum quantities allowed.

## Results

All data discussed in this paper are shown in Table I. Humoral immunosuppression of anti- $\mu$ -treated mice was in part established by the elimination of detectable serum IgM in all such animals (column 3). Serum IgG1, IgG2, and IgA levels were also determined, but have not been reported here because they simply confirmed the severe panspecific immunosuppression by anti- $\mu$  antibodies which has been repeatedly published elsewhere (1, 2, 4, 6). In addition to showing no detectable serum IgM, all anti- $\mu$ -treated mice demonstrated high circulating residuals of free anti- $\mu$  antibodies (column 4). The demonstration of these circulating antibodies underscores the very high levels of anti- $\mu$  treatment used. The overall degree of humoral immunosuppression in the anti- $\mu$ -treated mice was also reflected in their general failure to produce precipitating antibod-

TABLE I  
*IgM and Specific Antibody Levels of Mice Treated from Birth with PBS, NRS, or Anti- $\mu$  Antiserum and Subsequently Infected with *N. brasiliensis**

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5	Col. 6
Mouse no.	Injected with	Serum IgM level*	Free anti- $\mu$ level*	Anti-NRS precipitin level*	Anti-worm PCA‡
1	PBS	32	0	0	512
2	"	64	0	0	512
3	"	64	0	0	32
4	"	64	0	0	256
5	"	32	0	0	256
6	"	64	0	0	256
7	NRS	32	0	64	128
8	"	32	0	32	128
9	"	32	0	64	128
10	"	32	0	64	256
11	"	64	0	128	512
12	"	32	0	128	32
13	"	64	0	128	512
14	"	64	0	128	256
15	Anti- $\mu$	0	16	0	<4
16	"	0	16	0	-§
17	"	0	16	0	<4
18	"	0	16	0	0
19	"	0	16	4	0
20	"	0	8	0	0
21	"	0	8	0	0
22	"	0	8	0	0

\* Reciprocal serum dilution producing a precipitin band in a standardized gel diffusion test.

‡ PCA, passive cutaneous anaphylaxis test performed in rats.

§ Not available; test rat failed to give skin reaction with positive control.

ies against antigenic rabbit serum components (column 5), even though these animals received 28 or 29 injections of such antigens in the form of the suppressive serum itself. Only one suppressed mouse out of eight (no. 19) made any detectable anti-NRS precipitin response, and this was only a tiny fraction of that made by the NRS-treated control mice.

Measurement of PCA antibodies formed against *N. brasiliensis* during infection produced a distinct picture of IgE suppression by anti- $\mu$  antibodies (column 6). It was observed that every PBS and NRS control mouse thus infected produced a substantial reaginic response against the worms. In contrast, no anti- $\mu$ -treated mouse developed any detectable PCA response in this system. Achievement of active worm infection in the anti- $\mu$ -treated mice was verified by worm egg counts in the feces (11); such counts were similar in the PBS, NRS, and anti- $\mu$  groups after both worm infections (data not presented). This finding eliminates the possibility that failure of the anti- $\mu$  mice to make reaginic responses against the worms was due to a lack of appropriate antigenation by infection.

### Discussion

The uniform inhibition of PCA antibody formation in response to *N. brasiliensis* infection is a clear-cut demonstration of suppression of reaginic responsiveness by anti- $\mu$  antibodies. The likelihood that the reaginic antibodies involved

are in fact IgE is supported both by their fixation to rat skin and their heat lability (14); heating the reactive sera at 56°C for 90 min uniformly depressed their PCA activity below detectable limits, but did not reduce anti-NRS precipitin titers in any case (data not presented).

It is possible, of course, that the anti- $\mu$  serum used in these studies contained undetected traces of anti- $\epsilon$  activity which might be responsible for the observed suppression. That situation seems unlikely, however, in view of the degree of purity of IgM used to induce formation of the antiserum and the subsequent extensive absorption of this antiserum with other immunoglobulins. It seems more likely that the effect is in fact due to anti- $\mu$  antibodies, but that suppression of IgE responsiveness, like IgA suppression, requires a relatively high level of suppressive treatment. This could account for the lack of IgE suppression observed by Dwyer et al. (9). We believe, therefore, that the uniform anti- $\mu$ -mediated suppression of reaginic antibody formation against *N. brasiliensis* discussed here provides convincing evidence that IgE-forming cells, like those producing IgM, IgG1, IgG2, or IgA, arise from IgM-bearing precursors.

### Summary

Neonatally initiated injection of anti- $\mu$  antiserum in mice has been shown to suppress the formation of reaginic antibodies in response to infection with the intestinal nematode, *Nippostrongylus brasiliensis*. This observation supports the hypothesis that IgE-producing cells arise from IgM-bearing precursors.

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