

# Pathways of anaphylaxis in the mouse

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**Background:** Although anaphylaxis is classically mediated by IgE, FcεRI, mast cells, and histamine, several rodent studies suggest that an alternative pathway involving IgG, FcγRIII, macrophages and platelets, and platelet-activating factor (PAF) may be more important in the anaphylactic response to antigen challenge.

**Objectives:** We sought to determine the relative roles of the classical and alternative pathways of anaphylaxis in a mouse model characterized by mastocytosis and a high level of antigen-specific IgE antibody.

**Methods:** Wild-type, IgE-deficient, FcεRI-deficient, and mast cell-deficient mice were immunized with goat anti-mouse IgD antibody, which induces mastocytosis and a large IgE and IgG anti-goat IgG response, and then challenged 14 days later with antigen (goat IgG) or rat anti-mouse IgE mAb. Specific vasoactive mediators, cell types, Ig isotypes, or Ig receptors were blocked or eliminated before challenge in some experiments. The severity of anaphylaxis was gauged by changes in body temperature, physical activity, and mortality.

**Results:** Equal doses of antigen or anti-IgE mAb induced similar anaphylactic responses. Anti-IgE mAb-induced anaphylaxis was FcεRI and mast cell dependent and mediated predominantly by histamine. In contrast, neither mast cells nor platelets appeared important for antigen-induced anaphylaxis, which was FcγRIII and macrophage dependent and mediated predominantly by PAF.

**Conclusions:** Antigen-induced anaphylaxis in the mouse proceeds primarily through the IgG, FcγRIII, macrophage, and PAF pathway, even in an experimental model that is characterized by strong mast cell and IgE responses. The presence of FcγRIII on human macrophages makes it possible that the IgG, FcγRIII, macrophage, and PAF pathway also contributes to human anaphylaxis. (*J Allergy Clin Immunol* 2002;109:658-68.)

**Key words:** Anaphylaxis, mast cell, macrophage, allergy, histamine, platelet-activating factor, Fc receptors

## Abbreviations used

GaMD: Goat anti-mouse IgD antibody  
GIgG: Goat IgG  
5-HT: Serotonin  
MMCP: Mouse mast cell protease  
PAF: Platelet-activating factor

Anaphylaxis is an acute, life-threatening, allergic reaction in which a physiologic process that normally acts in a local and limited manner to protect against infection occurs massively and systemically. Although anaphylaxis is classically mediated by histamine released in response to antigen cross-linking of IgE bound to FcεRI on mast cells, both human and rodent studies indicate that this classical pathway does not account for all anaphylactic responses.<sup>1-4</sup> In particular, several rodent studies suggest that a pathway that involves IgG, FcγRIII, granulocytes, macrophages and platelets, and platelet-activating factor (PAF) may be more important than the classical pathway in animals challenged with antigen rather than with anti-IgE antibody.<sup>4-6</sup> These observations, concerns that mice may have poor IgE and mast cell responses and differences between mouse and human mast cell FcγRIII expression<sup>7</sup> and macrophage FcεRI expression,<sup>8,9</sup> raise questions about the applicability of mouse anaphylaxis studies to human subjects.

With this in mind, we have compared and analyzed the importance of mast cells, macrophages, granulocytes, platelets, IgE, FcεRI, FcγRII, FcγRIII, complement, histamine, serotonin (5-HT), and PAF in anaphylaxis induced by antigen or anti-IgE antibody in a mouse model in which a single injection of goat anti-mouse IgD antibody (GaMD) induces CD4<sup>+</sup> T-cell IL-3 and IL-4 production,<sup>10</sup> which promotes mastocytosis and large goat IgG (GIgG)-specific IgE and IgG antibody responses.<sup>11,12</sup> Results of our studies demonstrate that antigen-induced anaphylaxis depends on an FcγRIII, macrophage, and PAF-dependent pathway, even in this experimental system.

## METHODS

### Mice

Female BALB/c mice were purchased from NCI (Frederick, Md). IgE-deficient mice (FVBN background, a gift from P. Leder, Cambridge, Mass)<sup>13</sup> and FcεRIα-deficient mice (BALB/c background, a gift from J.-P. Kinet, Cambridge, Mass)<sup>14</sup> were bred in our animal facility along with the appropriate wild-type control animals. W/W<sup>v</sup> (mast cell-deficient) mice and litter mate control mice<sup>15</sup> were purchased from Jackson Labs (Bar Harbor, Me). All mice were age and sex matched within experiments and were used at 7 to 12 weeks of age.

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## Induction of anaphylaxis

All experiments were conducted with the approval of the Veteran's Administration Medical Center Institutional Animal Care and Use Committee. Mice (5 per group except where noted otherwise) were sensitized intravenously with 800  $\mu$ g of affinity-purified GaMD antibody in 200  $\mu$ L of normal saline or intraperitoneally with 200  $\mu$ L of GaMD antiserum.<sup>11,12</sup> Except where noted otherwise, mice were challenged 14 days later by means of intravenous injection of 100  $\mu$ g of IgG purified from normal goat serum (antigen), 100  $\mu$ g of rat IgG2a anti-mouse IgE mAb (EM-95),<sup>16</sup> or a rat IgG2b anti-mouse Fc $\gamma$ RII/III mAb (24G2),<sup>17</sup> which binds the low-affinity IgG receptors Fc $\gamma$ RII and Fc $\gamma$ RIII. Rectal temperatures were measured with a Digital Thermocouple Thermometer (Model BAT-12; Physitery Instruments Inc, Clifton, NJ) just before challenge, every 5 minutes for 30 minutes, and then every 15 minutes for the next 90 minutes. At the same time, mouse activity was assessed and quantitated on a 4-point scale (3, normal activity; 2, slow movement after prodding; 1, no movement in response to prodding; and 0, inability to right after being turned on side).

## Reagents

Hybridomas were obtained from the following sources: 24G2 (rat IgG2b anti-mouse Fc $\gamma$ RII/III mAb)<sup>17</sup> from ATTC (Rockville, Md); EM-95 (rat IgG2a anti-mouse IgE mAb)<sup>16</sup> from Zelig Eshhar (Rehoveth, Israel); RB6-8C5 (rat IgG2b granulocyte-depleting mAb)<sup>18</sup> from Robert Coffman, DNAX Corp (Palo Alto, Calif); and rat IgG2a (GL117) and rat IgG2b (J1.2) control mAbs from Dr John Abrams (DNAX). Monoclonal antibodies were produced as previously described.<sup>12</sup> PAF, 5-HT, triprolidine (an H-1 receptor-specific antihistamine), metergoline (a 5-HT<sub>1/2/7</sub> receptor antagonist), gadolinium chloride, neuraminidase type VI, and cyproheptadine (a combined H-1 receptor/5-HT<sub>1c/2</sub> receptor antagonist) were purchased from Sigma (St Louis, Mo). A PAF antagonist, CV-6209, was purchased from Biomol (Plymouth Meeting, Pa). A 5-HT<sub>2a</sub> receptor antagonist, ketanserin, was purchased from Alexis Biochemical (San Diego, Calif). Absorbed rabbit anti-mouse platelet antiserum was purchased from Accurate Chemicals (Westbury, NY), rabbit serum from Pel-Freez (Milwaukee, Wis), and cobra venom factor from Diamedix (Miami, Fla).

## Treatment of mice with inhibitors

All agents were diluted in normal saline to a final volume of 200  $\mu$ L per mouse unless otherwise stated. One hundred micrograms of anti-IgE mAb or 500  $\mu$ g of anti-Fc $\gamma$ RII/III mAb, respectively, was injected intraperitoneally 24 hours before challenge to deplete IgE or block Fc $\gamma$ RII/III. These treatments induce a mild form of anaphylaxis, from which mice rapidly recover, while depleting serum and cell-bound IgE or blocking Fc $\gamma$ RII and Fc $\gamma$ RIII for several days (data not shown).<sup>16,19</sup> Triprolidine, 200  $\mu$ g, was injected intraperitoneally 30 minutes before challenge to inhibit histamine. PAF was blocked with 66  $\mu$ g of CV-6209 injected intravenously 5 minutes before challenge. One hundred micrograms of metergoline in 1% carboxymethylcellulose or 60  $\mu$ g of ketanserin was injected intraperitoneally 30 minutes before challenge to selectively antagonize 5-HT. Macrophage function was inhibited by means of intravenous injection of 1 mg of gadolinium 24 hours before challenge.<sup>20</sup> Platelets were depleted by means of intravenous injection of absorbed rabbit anti-mouse platelet antiserum (1:40 vol/vol) or intraperitoneal injection of 0.1 U of neuraminidase<sup>21</sup> 24 hours before challenge. Two milligrams of anti-granulocyte mAb was injected intraperitoneally 48 hours before challenge to eliminate granulocytes.<sup>18</sup> Complement was depleted by means of intravenous injection of 2 U of cobra venom factor twice daily for 2 days before challenge.<sup>22</sup> Cyproheptadine, 0.4 mg, was injected intraperitoneally

30 minutes before challenge to inhibit both histamine and 5-HT. GL117, J1.2, and normal rabbit serum were used as controls.

## Measurement of histamine and PAF

Histamine and mouse mast cell protease 1 (MMCP-1) concentrations were measured with ELISA kits from IBL (Hamburg, Germany) and Moredun (Penicuik, Scotland), respectively. Mice were killed 15 minutes after challenge, and their spleens were removed immediately, snap-frozen in liquid nitrogen, and stored at  $-80^{\circ}$  C or on dry ice until assayed to measure PAF responses, as previously reported.<sup>23</sup>

## Platelet counts

Platelets were counted in at least 10 fields of Wright's stained blood smears at a magnification of 1250 $\times$ .

## Statistics

Spearman correlations between physical activity scores and rectal temperatures 30, 60, and 120 minutes after challenge were calculated with GraphPad Prism 2.0 software (San Diego, Calif). The Fisher exact method was used to assess the statistical significance of group differences in death rate, and the Mann-Whitney test was used to compare mediator concentrations between groups.

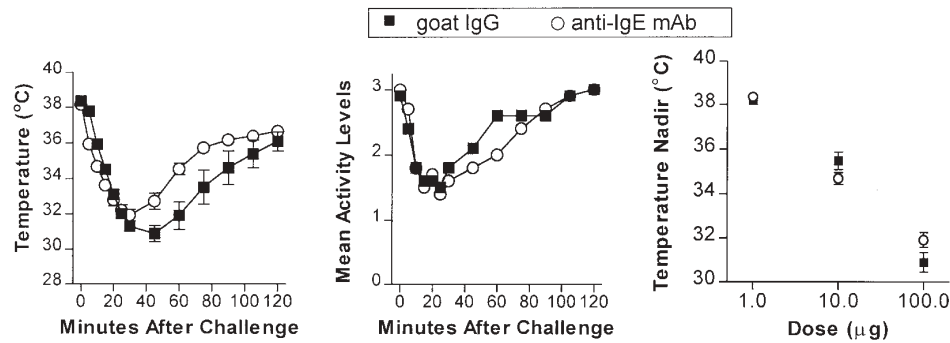
## RESULTS

### Anti-IgE mAb- and antigen-induced anaphylaxis have similar severities, kinetics, and sensitivities

Injection of mice with GaMD stimulates large IgG and IgE anti-GIgG antibody responses.<sup>24</sup> Challenge of GaMD-primed mice with either antigen (GIgG) or anti-IgE mAb intravenously 14 days after priming induces anaphylaxis. The severity, kinetics, and dose-response characteristics of anaphylaxis induced by injection of antigen or anti-IgE mAb were compared by evaluating decreases in mouse activity and rectal temperature for 2 hours after challenge (Fig 1, left and middle panels). Rectal temperature and activity decreased with similar rapidity and severity in mice challenged with either antigen or anti-IgE mAb. Decreases in rectal temperature and activity correlated closely in individual mice ( $r^2 = 0.65$ ). Similar doses of antigen or anti-IgE mAb were required to decrease rectal temperature (Fig 1, right panel).

### Ig isotypes and Ig Fc receptors involved in antigen- and anti-IgE mAb-induced anaphylaxis

The similar severities, kinetics, and dose-response characteristics of antigen- and anti-IgE mAb-induced anaphylaxis in GaMD-primed mice suggested that they might be mediated by the same mechanism. To test this hypothesis, we determined the IgE, Fc $\epsilon$ RI, and Fc $\gamma$ RII/III requirements for antigen- and anti-IgE mAb-induced anaphylaxis. IgE involvement was assessed in 2 ways. First, GaMD-primed mice were depleted of IgE and most likely mast cell desensitized by means of intraperitoneal injection of anti-IgE mAb 24 hours before intravenous challenge with antigen or anti-IgE mAb. Second, we compared responses to anti-IgE



**FIG 1.** Challenge with antigen or anti-IgE mAb induces similar decreases in activity and body temperature in GaMD-primed mice. BALB/c mice (10 per group) were injected intravenously with GaMD and then intravenously challenged 14 days later with 100  $\mu$ g of anti-IgE mAb or GIG. Rectal temperatures and activity levels were serially determined for 2 hours after challenge. Means and SEs for rectal temperatures (left panel) and means for activity level (middle panel) are shown. In a separate experiment GaMD-primed mice (5 per group) were challenged intravenously with 1, 10, or 100  $\mu$ g of either anti-IgE mAb or GIG. Means and SEs of lowest rectal temperature after challenge are shown for each dose (right panel). A repeat of all experiments provided similar results.

mAb or antigen in GaMD-primed wild-type, Fc $\epsilon$ RI-deficient, and IgE-deficient mice. Pretreatment with anti-IgE mAb completely inhibited anti-IgE mAb-induced anaphylaxis, as expected, but did not inhibit antigen-induced anaphylaxis (Fig 2, upper panels). Similarly, antigen, but not anti-IgE mAb, induced anaphylaxis in IgE-deficient mice (Fig 2, middle panels). Compatible results were obtained with Fc $\epsilon$ RI-deficient mice (Fig 2, lower panels), although some decrease in the severity of antigen-induced anaphylaxis was observed in the Fc $\epsilon$ RI-deficient mice in the experiment shown.

These low-affinity IgG receptors were blocked by means of intraperitoneal injection of anti-Fc $\gamma$ R2/3 mAb 24 hours before challenge to evaluate Fc $\gamma$ R2 and Fc $\gamma$ R3 involvement (Fig 2, upper panels). Pretreatment with anti-Fc $\gamma$ R2/3 mAb inhibited antigen-induced anaphylaxis but did not affect anti-IgE mAb-induced anaphylaxis (Fig 2, upper panels).

Taken together, these observations demonstrate that anaphylaxis induced by anti-IgE mAb requires IgE and Fc $\epsilon$ RI, but not Fc $\gamma$ R2 or Fc $\gamma$ R3, whereas antigen-induced anaphylaxis in our system is Fc $\gamma$ R2/3 dependent but requires neither IgE nor Fc $\gamma$ RI. Thus despite their similarities in general appearance, antigen- and anti-IgE mAb-induced anaphylaxis are mediated predominantly by different Ig isotypes and Fc receptors.

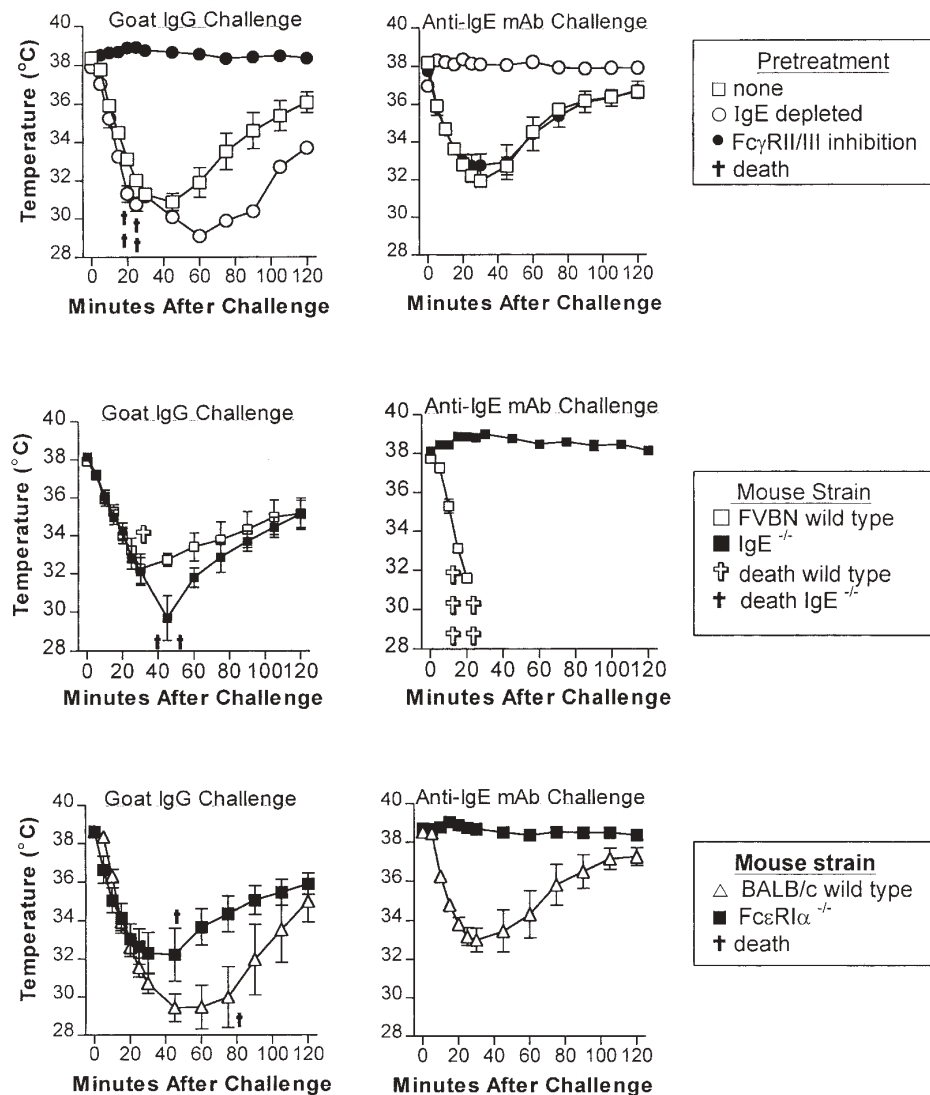
### Cells involved in anaphylaxis

Fc $\epsilon$ RI $\alpha$  involvement in anti-IgE mAb-induced anaphylaxis suggests that this type of anaphylaxis requires mast cells, basophils, or both, the only 2 cell types in the mouse that express the high-affinity IgE receptor.<sup>4,14</sup> Mast cells could also be required for antigen-induced, Fc $\gamma$ R2/3-dependent anaphylaxis because mouse mast cells express the stimulatory IgG Fc receptor Fc $\gamma$ R3.<sup>4,25</sup>

To determine mast cell participation in each type of anaphylaxis, we evaluated responses of GaMD-primed wild-type and mast cell-deficient W/W<sup>v</sup> mice to intravenous challenge with anti-Fc $\gamma$ R2/3 mAb, anti-IgE mAb, or antigen. Although anti-IgE mAb failed to induce anaphylaxis in mast cell-deficient mice, absence of mast cells did not inhibit anaphylaxis induced by means of intravenous injection of either antigen or anti-Fc $\gamma$ R2/3 mAb (Fig 3, upper panels). Consistent with these observations, mucosa mast cell degranulation, as measured by means of increased serum MMCP-1 levels,<sup>26,27</sup> was induced approximately 100-fold more by anti-IgE mAb than by antigen challenge ( $P < .01$ ), and anti-Fc $\gamma$ R2/3 mAb failed to induce any increase in MMCP-1 (Fig 3, lower panel). Thus mast cells are required for IgE-mediated anaphylaxis but have minimal involvement in antigen-induced anaphylaxis, and even though mouse mast cells express Fc $\gamma$ R3, ligation of this receptor does not appear to activate mucosa mast cell degranulation.

These observations indicated that cells other than mast cells must be involved in antigen-mediated anaphylaxis. Because previous studies have implicated granulocytes, macrophages, and platelets in anaphylaxis,<sup>5,28,29</sup> we evaluated whether any or all of these cell types are required for anaphylaxis in GaMD-primed mice. As expected, depletion of granulocytes, macrophages, or platelets failed to inhibit anti-IgE mAb-induced anaphylaxis (Fig 4, upper panels), and in fact, anti-granulocyte mAb pretreatment worsened the severity of anaphylaxis through an unexplained mechanism. In contrast, antigen-induced anaphylaxis was blocked by gadolinium depletion of macrophages and antibody depletion of platelets, but not by mAb depletion of granulocytes (Fig 4, lower panels).

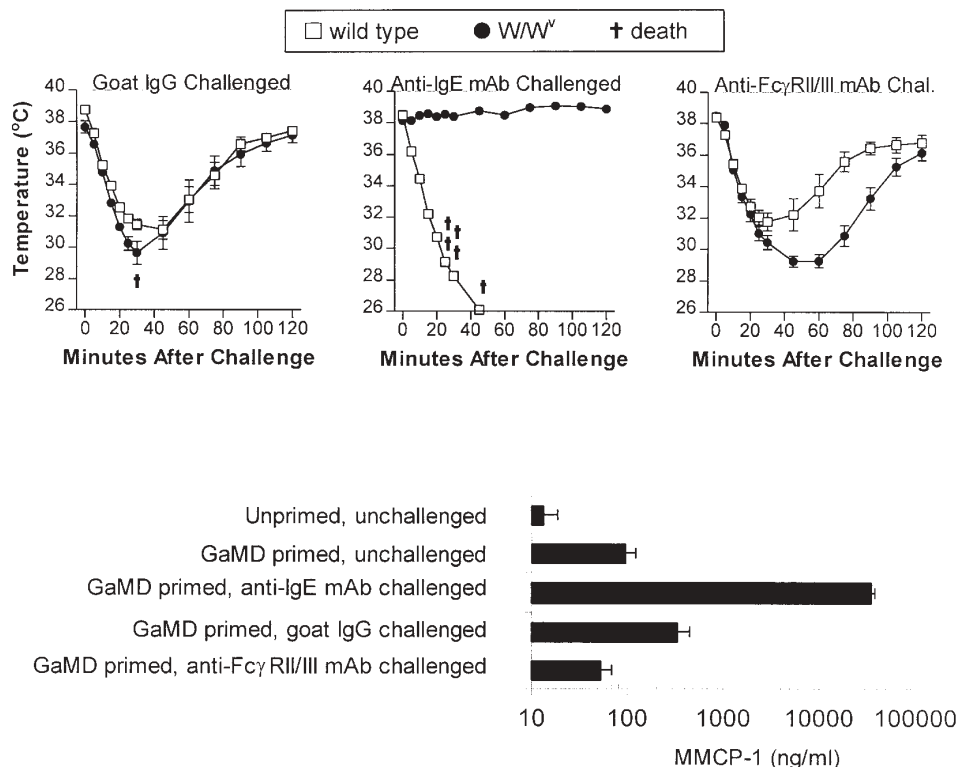
The suggestion that platelets are required for antigen-induced anaphylaxis posed a theoretic problem: mouse



**FIG 2.** Different Ig isotypes and Fc receptors are involved in antigen- and anti-IgE mAb-induced anaphylaxis. Mice in all experiments were primed with GaMD and intravenously challenged 14 days later with antigen or anti-IgE mAb. *Upper panels*, BALB/c mice (5 per group) were injected intraperitoneally 24 hours before challenge (day 13) with 100  $\mu$ g of anti-IgE mAb, 500  $\mu$ g of anti-FcγRII/III mAb, or an isotype-matched control mAb. Mice were challenged intravenously with 100  $\mu$ g of anti-IgE mAb or 10 mg of GlgG. *Middle panels*, IgE<sup>-/-</sup> and wild-type mice (5 per group) on the same FVB/N background were challenged intravenously with 100  $\mu$ g of anti-IgE mAb or 10 mg of GlgG. (A much higher dose of antigen is required to induce anaphylaxis in GaMD-primed FVB/N mice than in BALB/c mice.) *Lower panels*, FcεRIα<sup>-/-</sup> and wild-type mice on the same BALB/c background (5 per group) were challenged with 100  $\mu$ g of anti-IgE mAb or GlgG. Rectal temperatures (means and SEMs) are shown. Control mAbs had no effect on anaphylaxis (data not shown). A repeat of all experiments provided similar results.

platelets, unlike human platelets, lack a stimulatory FcγR.<sup>30,31</sup> This led us to investigate the possibility that a single dose of anti-platelet antibody might form sufficient antigen-antibody complexes with platelets to desensitize FcγRII/RIII signaling. Two experiments examined this possibility. First, GaMD-primed mice were injected daily with anti-platelet antiserum for 3 days before antigen challenge on the assumption that

platelet-IgG anti-platelet antibody complex levels would fall after the first day because of persistently low platelet counts. Second, mice were injected with neuraminidase, which decreases platelet levels without forming immune complexes.<sup>21</sup> Each treatment reduced average platelet counts by greater than 95% (data not shown) without inhibiting antigen-induced anaphylaxis (Fig 5) or anti-IgE mAb-induced anaphylaxis (data not shown). Taken



**FIG 3.** Mast cell dependence of anaphylaxis. *Upper panels*, Mast cell-deficient, W/W<sup>v</sup>, and wild-type litter mates (5 per group) were primed with GaMD and challenged intravenously with GlgG (*left panel*), anti-IgE mAb (*middle panel*), or anti-FcγRII/III mAb (*right panel*) 14 days later. Rectal temperatures (means and SEs) are shown. Repeat experiments provided similar results. *Lower panel*, BALB/c mice (5 per group) were left unprimed or primed with GaMD and challenged 14 days later as indicated. MMCP-1 levels in sera obtained 2 hours after challenge were determined by means of ELISA. MMCP-1 levels in anti-IgE mAb-challenged mice were higher than those in any other group ( $P < .01$ ), and MMCP-1 levels in GlgG-challenged mice were higher than those in unchallenged or anti-FcγRII/III mAb-challenged groups ( $P < .02$ ).

together, these observations demonstrate that antigen-induced anaphylaxis in our system requires macrophages but is mast cell, granulocyte, and platelet independent.

### Vasoactive mediators involved in anaphylaxis

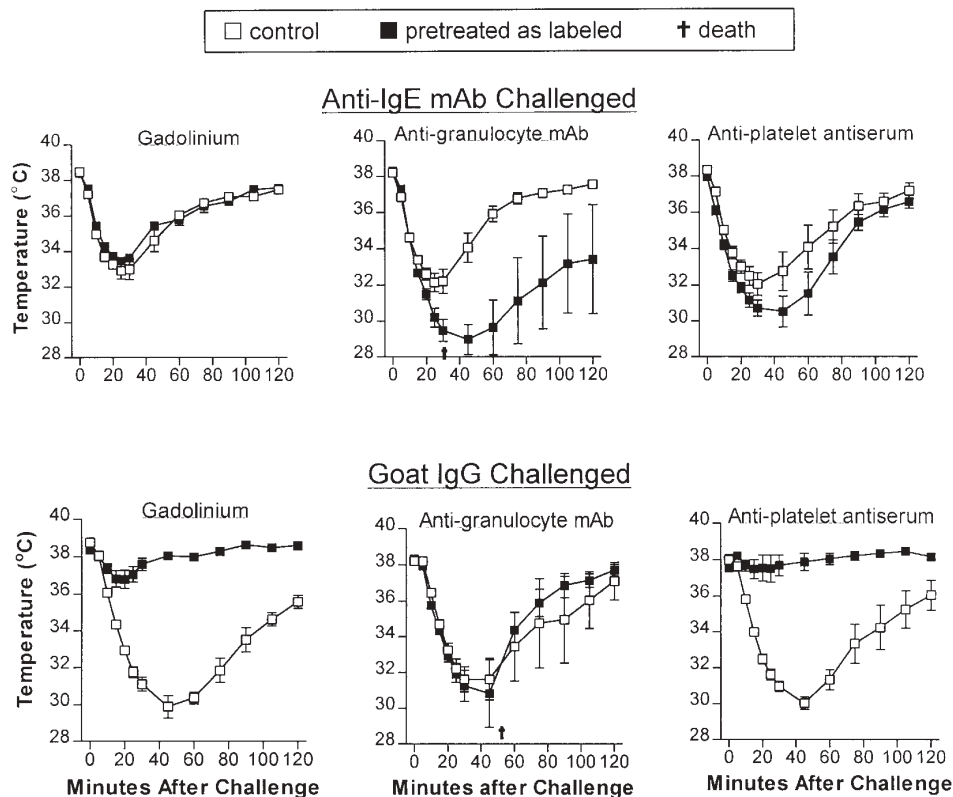
Histamine, PAF, and 5-HT have all been reported to be released during anaphylaxis, and each of these mediators can directly cause shock if injected into rodents in sufficient quantity.<sup>32,33</sup> Experiments were performed to determine the relative involvement of each of these mediators in anti-IgE mAb- and antigen-induced anaphylaxis. Pretreatment with the H1 antagonist triprolidine had little or no effect on antigen-induced anaphylaxis in mice primed with GaMD antibody (Fig 6, upper left panel) but strongly inhibited anti-IgE mAb-induced anaphylaxis (Fig 6, lower left panel). In contrast, antigen-induced anaphylaxis was strongly inhibited by the PAF antagonist CV 6209 (Fig 6, upper left panel) and by a second PAF antagonist (data not shown). CV 6209 also inhibited anti-

IgE mAb-induced anaphylaxis, although less markedly than triprolidine (Fig 6, lower left panel). This relatively slight inhibition by CV6209 was observed in several experiments. The 5-HT receptor antagonist metergoline had no effect on either antigen- or anti-IgE mAb-induced anaphylaxis (Fig 6, right panels) and neither did a second 5-HT receptor antagonist, ketanserin (data not shown).

Consistent with these observations and our MMCP-1 results (Fig 3), histamine levels were increased greater than 20-fold more in anaphylaxis triggered by anti-IgE mAb than in anaphylaxis triggered by antigen ( $P < .01$ ), whereas splenic PAF levels were increased in both antigen- and anti-IgE mAb-induced anaphylaxis (both  $P < .05$ , Fig 7).

Complement involvement in anaphylaxis was also assessed. Mice pretreated with cobra venom factor to deplete complement had no change in severity of anaphylaxis when challenged with either antigen or anti-IgE mAb (data not shown). Therefore both IgE- and IgG-mediated anaphylaxis are complement independent in our system.





**FIG 4.** Cell types involved in anaphylaxis. BALB/c mice (5 per group) were primed intravenously with GaMD and challenged intravenously 14 days later with either anti-IgE mAb (*upper panels*) or GlgG (*lower panels*). Some mice were pretreated with gadolinium (*left panels*), anti-granulocyte mAb (*middle panels*), or anti-platelet antiserum (*right panels*). Mean rectal temperatures and SEs are shown. Repeat experiments provided similar results. Normal rabbit serum and rat IgG2b mAb were used as controls and had no effect on anaphylaxis (data not shown).

### Anti-IgE mAb and antigen induce shock through the same target organs in GaMD-primed mice

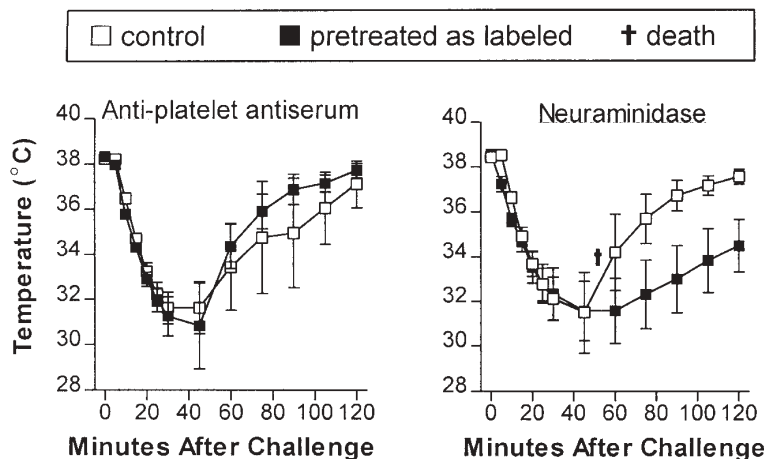
The observation that anti-IgE mAb- and antigen-induced anaphylaxis predominantly involve different Ig isotypes, receptors, cell types, and mediators raised the possibility that the target organs responsible for vascular collapse and the failure to maintain body temperature might also differ between the 2 types of anaphylaxis. If so, anaphylaxis induced by one mechanism might not desensitize mice to anaphylaxis induced by the other mechanism. To examine this possibility, we challenged GaMD-primed wild-type or mast cell-deficient W/W<sup>v</sup> mice with anti-IgE mAb, and 4 hours later, when temperatures of the challenged wild-type mice had returned to normal, we challenged these mice and mice that had never received anti-IgE mAb with antigen. Pretreatment with anti-IgE mAb had no effect on the subsequent response to antigen in W/W<sup>v</sup> mice, which do not experience anaphylaxis to anti-IgE mAb, but considerably ameliorated the response to antigen in wild-type mice (Fig 8). This indi-

cates that anti-IgE mAb-induced anaphylaxis temporarily desensitizes mice to antigen-induced anaphylaxis and suggests that the 2 types of anaphylaxis, although different in mechanism, probably act through a final common pathway that involves the same target organs.

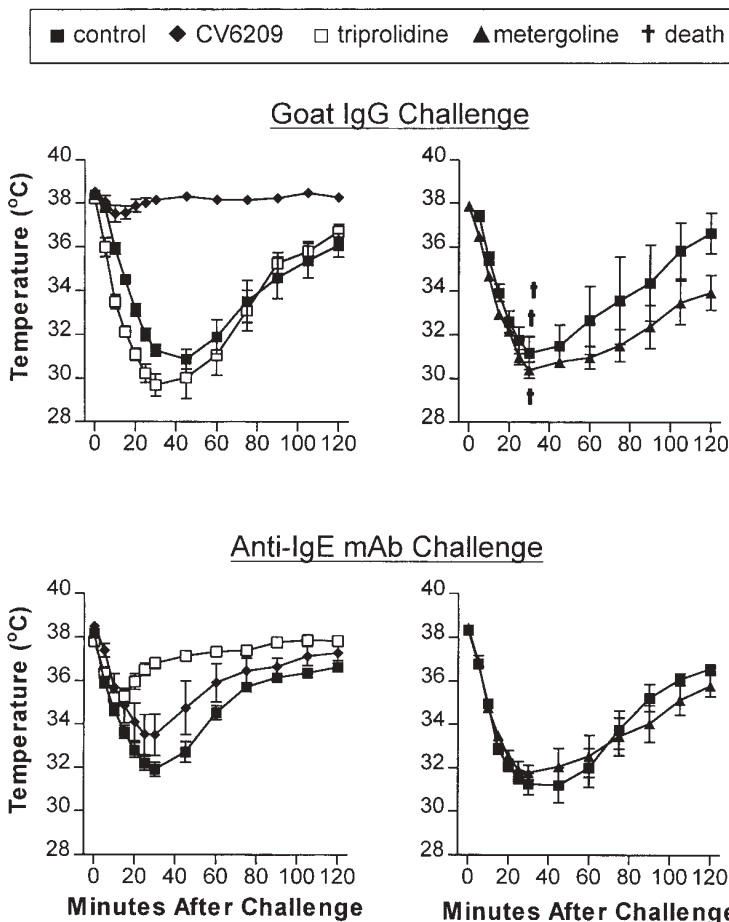
### DISCUSSION

Although anaphylaxis classically involves IgE, mast cells, and FcεRI, recent studies have demonstrated that none of these is required for the development of antigen-induced shock in previously immunized rodents. These studies also provided evidence for an alternative pathway in which IgG, non-mast cells (macrophages, granulocytes, and platelets), and FcγRIII are important.<sup>3-5,28,29,34-38</sup> We have examined the potential importance of each of these elements to determine whether antigen challenge would primarily induce the classical or the alternative pathway of anaphylaxis in a system that is characterized by large IgE and mast cell responses.

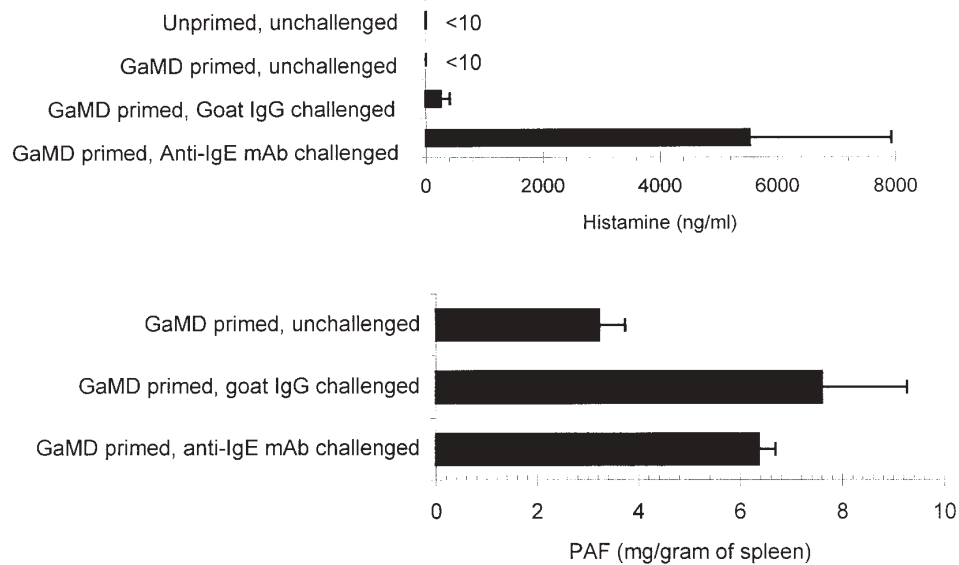
To do this, we immunized mice with GaMD, which activates mouse B cells by cross-linking their mIgD.



**FIG 5.** Platelets are not required for antigen-induced anaphylaxis. BALB/c mice (5 per group) were primed with GaMD and challenged with GlgG 14 days later. Some mice were injected daily with anti-platelet antiserum for 3 days before challenge (*left panel*) or 24 hours before challenge with neuraminidase (*right panel*). Means and SEs of rectal temperatures after challenge are shown. Normal rabbit serum had no effect on anaphylaxis (data not shown).



**FIG 6.** Mediators involved in anti-IgE and antigen-induced anaphylaxis. BALB/c mice (5 per group) were GaMD primed and then challenged 14 days later with either GlgG (*upper panels*) or anti-IgE mAb (*lower panels*). Where indicated, mice were desensitized before challenge with an antihistamine (triprolidine), a PAF antagonist (CV6209), or a 5-HT antagonist (metergoline). Rectal temperatures (means and SEs) are shown for 2 hours after challenge. Repeat experiments provided similar results. Experiments that substituted the 5-HT antagonist ketanserin for metergoline provided similar results. Combinations of antagonists (eg, CV6209 and triprolidine, metergoline and CV6209, or metergoline and triprolidine) did not consistently have greater inhibitory effects than the most potent antagonist given alone (data not shown).



**FIG 7.** Histamine and PAF responses to anti-IgE mAb and antigen challenge. BALB/c mice were primed with GaMD and challenged 14 days later with either antigen or anti-IgE mAb. Mice (5 per group) were bled 2 minutes after challenge, and plasma histamine levels were determined by means of ELISA. Other groups were killed 15 minutes after challenge, and spleens were immediately removed and flash frozen in liquid nitrogen. Splenic PAF levels were determined as described in the "Methods" section. Means and SEMs are shown. Histamine levels differ significantly between anti-IgE mAb-challenged and all other groups ( $P < .01$ ) and between antigen-challenged and all other groups ( $P < .01$ ). PAF levels differ significantly between the unchallenged group and each of the 2 challenged groups ( $P < .01$ ).

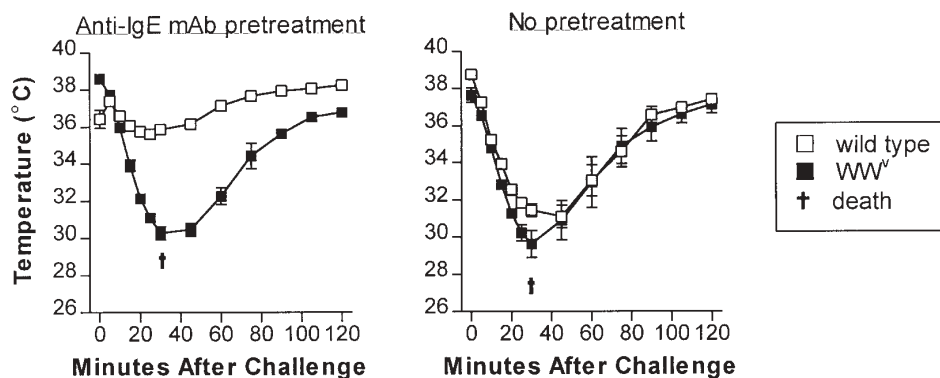
These activated B cells process and present GIgG to GIgG peptide-specific CD4<sup>+</sup> T cells, which respond by secreting cytokines that induce mastocytosis and by helping B cells to mount a massive IgG1 and IgE response.<sup>10,11,24,39,40</sup> Approximately 10% to 20% of this antibody response is GIgG specific.<sup>24</sup> We expected that this system would be useful for demonstrating antigen induction of the classical pathway of anaphylaxis.

Indeed, the classical pathway could easily be demonstrated in GaMD-immunized mice by challenging them 14 days after immunization (when serum IgE levels had returned to baseline but IgE remained bound to mast cell FcεRI<sup>11</sup>) with an mAb that efficiently cross-links IgE. As expected, anti-IgE mAb-induced anaphylaxis was dependent on IgE (Fig 2), FcεRI (Fig 2), and mast cells (Fig 3), but not on macrophages, granulocytes, platelets (Figs 4 and 5), or complement (data not shown). A significant role for histamine in anti-IgE mAb-induced anaphylaxis was demonstrated by a large increase in serum histamine levels 2 minutes after anti-IgE mAb challenge (Fig 7) and marked inhibition of anaphylaxis by an H1 antagonist (Fig 6). It is possible that even more complete inhibition would have been achieved had mice been pretreated with both H1 and H2 antagonists. PAF also had a role in anti-IgE mAb-induced anaphylaxis: Anti-IgE mAb-induced anaphylaxis was partially inhibited by a PAF antagonist, and splenic PAF levels increased after anti-IgE mAb challenge; however, PAF was quantitatively less important than histamine in the anti-IgE anaphylaxis pathway.

Anaphylaxis induced by challenging GaMD-primed mice with antigen (GIgG) induced a response that was indistinguishable in its kinetics, severity, target organ involvement, and challenge dose requirement from that induced by anti-IgE mAb (Figs 1 and 8) but differed remarkably in its physiologic mechanism. Antigen-induced anaphylaxis was IgE, FcεRI, and mast cell independent (no inhibition in IgE-deficient, IgE-depleted mice; FcεRI-deficient mice; or mast cell-deficient mice, respectively; Figs 2 and 3). In addition, although histamine is the most important vasoactive mediator in anaphylaxis induced by anti-IgE mAb, it had little role in the anaphylactic response to antigen challenge, as demonstrated by the lack of inhibition of anaphylaxis by antihistamine (Fig 6). This does not indicate that there is no mast cell degranulation and histamine release after antigen challenge in GaMD-primed mice; both, in fact, occur (Figs 3 and 7). Rather, it is likely that the amount of mast cell degranulation and histamine release that occur through an antigen-triggered, IgE/FcεRI-dependent mechanism is insufficient in our system to induce detectable shock.

In contrast, FcγRIII, macrophages, and PAF are critical in our system for antigen-induced anaphylaxis; anaphylaxis was blocked completely or almost completely by pretreating mice with anti-FcγRII/RIII mAb (Fig 2), inhibiting macrophage function with gadolinium (Fig 4), or blocking PAF effects with CV6209 or a second inhibitor (Fig 6 and data not shown). It is likely that macrophages contribute to anaphylaxis in this pathway by producing





**FIG 8.** Anti-IgE mAb-induced anaphylaxis temporarily desensitizes mice to antigen-induced anaphylaxis. Mast cell-deficient  $W/W^v$  mice (3 or 4 per group) or wild-type mice (4 or 5 per group) on the same genetic background were primed with GaMD and received no further treatment or were injected 14 days later intraperitoneally with anti-IgE mAb. Four hours after anti-IgE mAb injection, all mice were challenged intravenously with GlgG. Rectal temperatures (means and SEs) after GlgG challenge are shown.

PAF and that gadolinium inhibits antigen-induced anaphylaxis by interfering with PAF production because gadolinium treatment had no effect on shock induced directly by means of PAF injection (data not shown).

Our results differ from those of previously reported studies of antigen-induced anaphylaxis by failing to identify an important role for granulocytes or platelets.<sup>28,29</sup> Instead, our observations suggest that inhibition of anaphylaxis in mice injected with antibodies to either cell type was probably caused by the formation of immune complexes of anti-granulocyte or anti-platelet antibodies with granulocyte or platelet antigen, respectively, which most likely bind to  $Fc\gamma RIII$  on macrophages and temporarily desensitize these cells.

Our results also differ from those of previous studies that suggested that 5-HT release contributes substantially to antigen-induced anaphylaxis in the mouse. We found no evidence for decreased severity of antigen- or anti-IgE mAb-induced anaphylaxis in mice treated with any of 3 different 5-HT antagonists (Fig 6 and data not shown), yet the effects of a lethal dose of 5-HT could be completely inhibited by these 5-HT antagonists (data not shown). Similarly, in experiments in which complement was depleted by treating mice with cobra venom factor, we found no evidence that complement contributes substantially to anaphylaxis in our system (data not shown).

Our observations bear on the possibility that IgG may be involved in human anaphylaxis. Although mast cell degranulation that is induced by IgG1-bound  $Fc\gamma RIII$  can induce passive cutaneous anaphylaxis in the mouse, and IgE binding to  $Fc\gamma RIII$  has also been proposed to promote systemic anaphylaxis in this species, our studies fail to provide evidence that mast cell  $Fc\gamma RIII$  expression or that IgE binding to  $Fc\gamma RIII$  contributes substantially to systemic murine anaphylaxis. Anaphylaxis induced by either antigen or anti- $Fc\gamma RII/III$  mAb challenge in GaMD-primed mice was not significantly affected by mast cell depletion, and challenge with anti-

$Fc\gamma RII/III$  mAb failed to stimulate MMCP-1 release (Fig 3). Furthermore, anti-IgE mAb failed to induce anaphylaxis in  $Fc\epsilon RI$ -deficient mice (Fig 2), even though it theoretically could have reacted with IgE bound to mast cell  $Fc\gamma RIII$ . These negative observations suggest that differences between mouse and human models that might theoretically affect anaphylaxis, such as the presence of  $Fc\gamma RIII$  on mouse, but not human, mast cells and the ability of IgE to bind to mouse, but not human,  $Fc\gamma Rs$ , have little relevance for systemic anaphylaxis in either species. In fact, the presence of  $Fc\epsilon RI$  on human, but not mouse, macrophages raises the possibility that macrophages may be even more important for human than for mouse anaphylaxis.

These considerations raise 2 related issues. First, if IgG-mediated anaphylaxis is relevant to human subjects, why has it not been as apparent as it is in the mouse? Second, if GaMD-primed mice have high levels of mast cell-bound, GlgG-specific IgE, why is the classical pathway of anaphylaxis of so little importance in antigen-induced anaphylaxis in our system? We hypothesize that both issues are explained to a considerable extent by the very high levels of antigen-specific serum IgG that are present in GaMD-immunized mice and in many other rodent models of anaphylaxis but may not be present in most human subjects with allergy. The binding of allergen by such high levels of IgG antibody in mouse serum might both create sufficient IgG-allergen complexes to trigger macrophage PAF production through  $Fc\gamma RIII$  and neutralize allergen before it can cross-link  $Fc\epsilon RI$ -bound allergen-specific IgE on mast cells. A prediction made by this hypothesis, that increasing the amount of GlgG used to challenge GaMD-primed mice would increase the mast cell contribution to anaphylaxis by allowing antigen to reach mast cell-bound IgE, is consistent with a recent study in our laboratory in which anti- $Fc\gamma RII/III$  mAb failed to block anaphylaxis when mice were challenged with 10  $\mu$ g, instead of 100  $\mu$ g, of GlgG. The same study

demonstrated increased evidence of mast cell degranulation in mice challenged with the higher antigen dose (R. Strait and F. Finkelman, manuscript in preparation). Additional studies to determine whether lower doses of antigen will trigger clinically relevant mast cell degranulation in IgG-deficient mice are in progress.

If correct, our hypothesis raises the possibility that hyperimmunization of patients with allergies might block IgE-mast cell-mediated symptoms but promote activation of the alternative IgG-macrophage-mediated anaphylaxis pathway by allergens that enter the body through a parenteral route. This consideration is consistent with observations that not all human patients experiencing anaphylaxis have detectable antigen-specific IgE antibodies or evidence of mast cell degranulation and may be particularly relevant for planned studies in which pretreatment of allergic patients with an anti-IgE mAb would allow immunization with higher doses of allergen that might stimulate larger IgG antibody responses. These considerations would be less relevant for patients who have anaphylaxis to ingested allergens because there is little IgG antibody in the intestinal lumen that could block binding of allergen to IgE on intestinal mucosa mast cells.

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