population crashes (*7*). Reducing the parasite burdens reduced the variance in the population growth rate and produced an apparent reduction in the decline of the treated populations (Fig. 2). This experiment illustrated that parasitic nematodes were necessary for the cyclic declines in abundance that were observed in grouse populations. In both populations that were treated twice (Fig. 2C) and in one of the populations that was treated once (Fig. 2B), the effect of the treatment was apparent in comparison with the controls, although the results are less clear in the remaining population, which was treated just once. We suspect this was because the keeper treated a relatively low proportion of the grouse population (~15%). Even with these results, the findings were still significant and demonstrate that parasites played a key role in causing population cycles.

To determine the effectiveness of the treatment, we calculated the proportion of the population that should be treated in order to prevent a population crash. We addressed this problem with a modified form of the general macroparasite model (*5*) that incorporates the experimental procedures of direct oral treatment (*8*) (Table 1). Individuals in the model were classified as either untreated (with natural levels of infection) or treated (with no parasites). Treatment of a proportion (p) of the population was triggered in the model whenever the growth rate of the parasite population increased (becomes positive). The worms in the treated grouse suffered an increased mortality rate, so their life expectancy was <1 week, whereas the remaining untreated birds ($1 - p$) continued to release infective stages into the environment, which infected both treated and untreated hosts. Numerical solutions of the model's dynamics showed that treatment of >20% of the hosts was sufficient to prevent the cyclic crashes in host density (Fig. 3) and provided a good explanation for all the results of the experiment.

The results from this study show that population cycles in red grouse are the result of a single trophic interaction between a parasite and its host. Combined with the modified macroparasite model, these results show that parasites were both sufficient and necessary in causing cycles in these populations. They also show that intrinsic mechanisms do not need to be evoked as a cause of cyclic fluctuations in grouse abundance (*9*). Previous studies have undertaken detailed experiments at a lower spatial scale. For example, a factorial manipulation of the food and predators of snowshoe hares on 1-km^2 plots indicated that at least three trophic levels of interaction are involved in producing cycles (*3*). Nevertheless, to the best of our knowledge, this is the first time that manipulations of a mechanism in a cyclic species have demonstrated the cause of population cycles on a large scale.

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7. Variance in the rate of population change ($\ln N_{t+1} - \ln N_t$) was significantly greater in control populations than in populations that were treated once ($F = 2.74$, $P < 0.05$) and in populations that were treated twice ($F = 36.5$, $P < 0.001$), but there was no difference between control populations ($F = 1.07$, $P > 0.2$).
8. The dynamics of the parasitic nematode and the grouse were modeled with six coupled differential equations. These describe changes in treated hosts (H_T), untreated hosts (H_U), the adult parasite population in these two classes of host (P_T and P_U, respectively), the free-living stages of the parasite (eggs and larvae) (W), and the chicks produced by the hosts (C). The parameter values and definitions are given in Table 1.

$$dH_U/dt = (1-p)\theta C - [b + \Delta(H_U + H_T)]H_U - \alpha P_U \quad (1)$$

$$dC/dt = a(H_U + H_T) - \delta(P_U + P_T) - (b + \theta)C \quad (2)$$

$$dP_T/dt = \beta H_T W - \{[\mathrm{if}(dP/dt)>0,\mu,c,\mu] + b + \alpha\} - \alpha\frac{P_T^2}{H_T}\frac{k+1}{k} \quad (3)$$

$$dP_U/dt = \beta H_U W - (\mu + b + \alpha)P_U - \alpha\frac{P_U^2}{H_U}\frac{k+1}{k} \quad (4)$$

$$dW/dt = \lambda(P_U + P_T) - [\gamma + \beta(H_U + H_T)]W \quad (5)$$

$$dH_T/dt = p\theta C - [b + \Delta(H_U + H_T)]H_T - \alpha P_T \quad (6)$$

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Interleukin-13: Central Mediator of Allergic Asthma

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The worldwide incidence, morbidity, and mortality of allergic asthma are increasing. The pathophysiological features of allergic asthma are thought to result from the aberrant expansion of CD4$^+$ T cells producing the type 2 cytokines interleukin-4 (IL-4) and IL-5, although a necessary role for these cytokines in allergic asthma has not been demonstrable. The type 2 cytokine IL-13, which shares a receptor component and signaling pathways with IL-4, was found to be necessary and sufficient for the expression of allergic asthma. IL-13 induces the pathophysiological features of asthma in a manner that is independent of immunoglobulin E and eosinophils. Thus, IL-13 is critical to allergen-induced asthma but operates through mechanisms other than those that are classically implicated in allergic responses.

Recent decades have brought dramatic increases in the prevalence and severity of allergic asthma. In the United States, 15 million people are currently thought to suffer from the disorder (*1*). Allergic asthma is characterized by airway hyperresponsiveness (AHR) to a variety of specific and nonspecific stimuli, chronic pulmonary eosinophilia, elevated serum immunoglobulin E (IgE), and excessive airway mucus production (*2*). The pathophysiology of asthma is thought to be mediated by CD4$^+$ T lymphocytes producing a type 2 cytokine profile: (i) CD4$^+$ T cells are necessary for the induction of allergic asthma in murine models; (ii) CD4$^+$ T cells producing type 2 cytokines undergo expansion in these models and in patients with allergic asthma; and (iii) the amount of type 2 cytokines is increased in the airway tissues of asthmatics and animal models (*3–5*). The circumstantial evidence for the importance of IL-4 and IL-5, which are paradigmatic type 2 cytokines, has been compelling (*6–8*). However, although an antibody-mediated blockade of IL-4 during allergen sensitization ablates the development of allergic asthma, a similar blockade of IL-4 before or during an antigen challenge inhibits neither allergic in-

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flammation nor AHR (*9*). Thus, IL-4 generates T helper cell 2 (T_H2) deviation in these models (*10*) but is not necessary for the expression of allergic asthma. The $CD4^+$ T cell–derived factor or factors that mediate allergic asthma remain elusive.

IL-13 is a T_H2 cytokine that binds to the α chain of the IL-4 receptor (*11*). We therefore examined the role of IL-13 in allergic asthma. A well-characterized murine model of allergic asthma was used, in which allergen exposure results in AHR, pulmonary eosinophilia, increases in antigen-specific serum IgE amounts, and increases in airway epithelial mucus content (*12*). Male A/J mice were immunized intraperitoneally and were subsequently challenged intratracheally with soluble ovalbumin (OVA); the allergic phenotype was assessed 4 days after the antigen challenge (*13*). Blockade of IL-13 was performed by the systemic administration of a soluble IL-13α2-IgGFc fusion protein (sIL-13Rα2-Fc), which specifically binds to and neutralizes IL-13, 24 hours before subsequent intratracheal allergen challenges (*14*). Antigen challenge of allergen-immunized mice resulted in significant increases in airway responsiveness to acetylcholine (*15*) (Fig. 1A). Blockade of IL-13 resulted in a complete reversal of such allergen-induced AHR; thus, IL-13 is necessary for the expression of AHR in this model. The ability of IL-13 ablation to reverse AHR after the full development of the phenotype of allergic asthma contrasts with the inability of IL-4 ablation to accomplish such a reversal. The mechanism underlying the effectiveness of IL-4Rα blockade in reversing allergen-induced AHR (*12*) may be the inhibition of IL-13–mediated processes, which is consistent with the fact that Stat6 activation is downstream of IL-4Rα–mediated signaling for both cytokines. IL-13 is probably the primary $CD4^+$ T cell–derived factor responsible for allergen-induced AHR.

To evaluate the candidate mechanisms underlying IL-13–dependent expression of AHR, we characterized known allergic effector cascades. Eosinophils have been implicated as primary effector cells in asthma and asthmatic AHR (*16*), but the inhibition of IL-13 before repeat antigen provocation did not significantly affect allergen-induced pulmonary eosinophilia (*17*) (Fig. 1B). To assess the relevance of IgE-mediated pathways, we measured OVA-specific serum IgE (*18*).

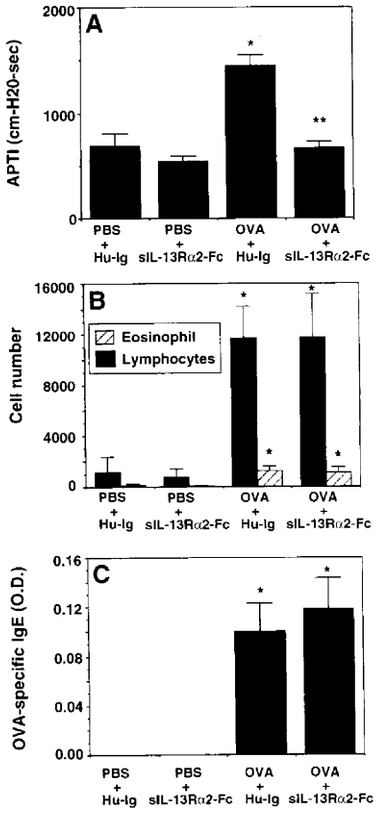

Fig. 1. Reversal of allergen-induced AHR by the in vivo blockade of IL-13. Ten days after the initial intratracheal challenge, OVA- and PBS-immunized mice were again challenged intratracheally with either OVA or PBS. Mice were given sIL-13Rα2-Fc (400 μg) or an equivalent amount of control human Ig (Hu-Ig) by intraperitoneal injection on days −1, 0, +1, and +3 of the secondary antigen challenge. The allergic phenotype was assessed 4 days after the PBS or OVA challenge. (**A**) AHR to the acetylcholine challenge, defined by the time-integrated rise in peak airway pressure [airway-pressure-time index (APTI) in centimeters of H_2O × seconds]. (**B**) Inflammatory cell composition of BAL fluids. Cell differential percentages were determined by light microscopic evaluation of cytospin preparations. Data are expressed as absolute numbers of cells. (**C**) OVA-specific serum IgE concentrations. In (A) through (C), the results are means ± SEM (error bars) of 8 to 10 animals per group and are representative of two independent experiments. $*P < 0.05$, compared with the respective PBS control groups; $**P < 0.05$, compared to the OVA+Hu-Ig group [one-way analysis of variance (ANOVA) followed by Fisher's least significant difference test for multiple comparisons].

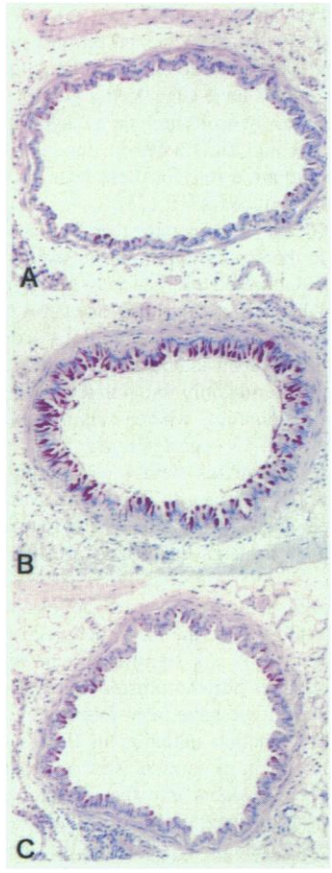

Fig. 2. Effects of the IL-13 blockade on allergen-driven increases in mucus-containing cells in the airway epithelium. Lung sections (four per experimental group and four sections per animal) were fixed in formalin; cut into 10-μm sections; and stained with hematoxylin, eosin, and periodic acid–Schiff. Representative sections are shown. (**A**) PBS+Hu-Ig section showing PBS-immunized and PBS-challenged controls and few mucus-containing cells. (**B**) OVA+Hu-Ig section showing allergen-induced increases in interstitial inflammatory cells and increases in the number of goblet cells containing mucus. (**C**) OVA+sIL-13Rα2-Fc section showing the inhibitory effect of the IL-13 blockade on allergen-induced mucus production in goblet cells. These data are representative of two independent experiments.

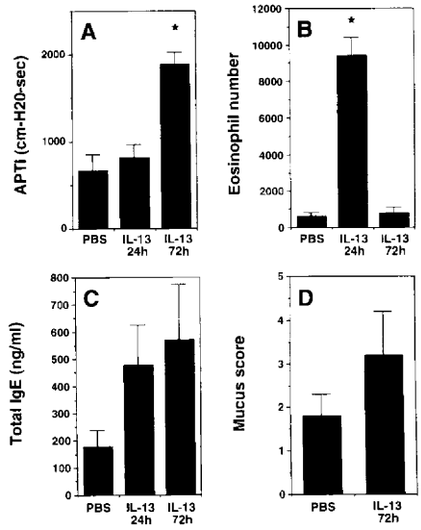

Fig. 3. IL-13 induction of airway hyperreactivity. Naïve mice were given murine rIL-13 (5 μg per mouse in a total volume of 50 μl) or PBS daily by intratracheal instillation. Twenty-four and 72 hours after the last treatment, (**A**) AHR, (**B**) BAL eosinophil numbers, and (**C**) serum total IgE concentrations were determined; (**D**) the mucus score was determined 72 hours after treatment. In (A) through (D), the results are means ± SEM (error bars) of 7 to 10 animals per group and are representative of three independent experiments. $*P < 0.05$, compared to the PBS group (Student's t test).

OVA-specific IgE was observed in OVA-sensitized and OVA-challenged mice, whereas no antigen-specific antibody was detected in phosphate-buffered saline (PBS)–immunized and PBS-challenged mice (Fig. 1C). Blockade of IL-13 did not alter OVA-specific IgE concentrations—a lack of suppression that is likely due to the fact that the IL-13 blockade occurred after initial antigen priming and antibody formation. Nonetheless, these results show that AHR is not dependent on IgE production in this model, which is consistent with a report that allergic AHR develops normally in IgE-deficient mice (*19*).

In congruence with the pathology of human asthma, allergic asthma in murine models is associated with a substantial increase in the mucus content of the airway epithelium (*7, 12*). Mucus hypersecretion is particularly profound in autopsy specimens from patients who die of acute asthma attacks (*20*). Blockade of IL-13 reverses allergen-induced increases in mucus-containing cells in the airways (Fig. 2), demonstrating that allergen-induced increases in airway mucus content are dependent on IL-13. IL-4 has also been implicated in this process, because IL-4 transgenic mice display goblet-cell hyperplasia in the absence of antigen sensitization (*7*). However, the transfer of T_H2 clones from IL-4–deficient mice into murine airways induces mucus overproduction (*21*), which suggests that the immunoregulatory role of IL-4 should be carefully differentiated from its role as an effector molecule.

If IL-13 is necessary for the expression of allergic AHR, is it sufficient to induce it? The daily administration of recombinant IL-13 (rIL-13) to the airways of naïve (unimmunized) mice induced AHR, demonstrating that increases in IL-13 activity were sufficient to induce AHR (Fig. 3A) (*22*). AHR developed within 72 hours from the start of rIL-13 administration. A significant influx of eosinophils into bronchoalveolar lavage (BAL) fluid was observed soon after rIL-13 administration; however, pulmonary eosinophilia was not observed at the time of expression of AHR (Fig. 3B). Although the importance of the time course of eosinophil influx remains unclear, it suggests that IL-13 alone may be sufficient to initiate eosinophilic infiltration of the airways, perhaps through its ability to up-regulate chemokine expression (*23*). Airway administration of rIL-13 also resulted in a time-dependent increase in total serum IgE (Fig. 3C) (*24*), which is in line with the ability of IL-13 to regulate IgE synthesis (*25*). Increases in serum IgE were independent of any immunization with allergen; these findings are consistent with the observation that the human asthmatic phenotype correlates better with total, rather than allergen-specific, serum IgE concentrations (*26*). As predicted from our IL-13 inhibition studies, the administration of rIL-13 induced an increase in airway mucus production (Fig. 3D) (*27*).

Although IL-13 thus appears capable of inducing the entire allergic asthmatic phenotype, the results of the IL-13 blockade experiments clearly show that IL-13–dependent AHR occurs by mechanisms that are independent of IgE and eosinophils in this model. The exact mechanism or mechanisms by which IL-13 induces AHR are currently unknown. The delayed time course for AHR induction suggests that IL-13 does not directly cause airway smooth muscle constriction. Reasonable hypotheses include direct time-dependent alterations in smooth muscle function (IL-13 receptors have yet to be demonstrated on airway smooth muscle) and indirect effects that are achieved through mediators released by surrounding cells. Although recent studies have suggested a possible role for sensory neuron–derived tachykinins in AHR, preliminary studies in our laboratory do not support a role for these neuropeptides in IL-13–induced AHR (*28*).

Our data demonstrate a critical role for IL-13 in the expression of murine asthma and suggest that, although IL-4 may be of immunoregulatory importance, IL-4 is not a prime effector molecule. These findings may be relevant to human asthma. Overexpression of IL-4 is predominantly found in the airways of allergic asthmatics, whereas significant elevations in IL-13 expression are found in the airways of patients with both allergic and nonallergic asthma (*4, 29*). Human asthma has been linked to a region of chromosome 5q, which contains the genes for both IL-4 and IL-13 (*30*). Although polymorphisms in the IL-13 gene have yet to be examined, polymorphisms in the IL-4 gene are well-described (*31*). No significant correlations between such polymorphisms and the asthmatic phenotype have been found; however, a gain-of-function mutation in IL-4Rα was recently shown to be associated with asthma (*32*). These insights into the immunopathogenesis of allergic asthma should provide direction for the development of therapeutics for this increasingly prevalent disease.

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13. Six-week-old male A/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and were housed under laminar flow hoods in an environmentally controlled specific pathogen-free animal facility for the duration of experiments (there were 4 to 10 mice per experimental group). The studies reported here conformed to the principles for laboratory animal research, as outlined by the Animal Welfare Act and the Department of Health, Education, and Welfare (NIH) guidelines for the experimental use of animals. Mice were immunized by an intraperitoneal injection of OVA (10 μg; crude grade IV) (Sigma) in PBS (0.2 ml) or of PBS alone. Fourteen days after immunization, mice were anesthetized with a mixture of ketamine and xylazine [45 and 8 mg per kilogram of body weight (mg/kg), respectively] and challenged intratracheally with 50 μl of a 1.5% solution of OVA or an equivalent volume of PBS as a control. Ten days after this first antigen challenge, mice were challenged again intratracheally with either OVA or PBS. Characterization of the allergic phenotype was performed 96 hours after the second antigen challenge.
14. For soluble expression of the murine IL-13Rα2, a pED expression vector containing DNA encoding the murine sIL-13Rα2 extracellular domain, fused in-frame with the heavy chain constant domains 2 and 3 of human IgG1, was transfected into CHO cells [D. D. Donaldson *et al.*, *J. Immunol.* **161**, 2317 (1998)]. The sIL-13Rα2-Fc was purified with protein A–Sepharose (*33*). The in vitro median inhibitory dose, as determined by its ability to neutralize 3 ng/ml of murine IL-13 in the B9 proliferation assay was ~10 ng/ml. Human Ig, used as a control for sIL-13Rα2-Fc, was similarly purified by protein A–Sepharose chromatography from a 10% solution of human immune globulin that is commercially available for intravenous administration (Miles, Berkeley, CA) (*33*). Mice were given sIL-13Rα2-Fc (400 μg) or an equivalent amount of the control human Ig by intraperitoneal injection on days −1, 0, +1, and +3 of the secondary antigen challenge.
15. Airway reactivity to the intravenous administration of acetylcholine was measured (*12*). Three days after the final intratracheal challenge, mice were anesthetized with sodium pentobarbital (90 mg/kg), intubated, ventilated at a rate of 120 breaths/min with a constant tidal volume of air (0.2 ml), and paralyzed with decamethonium bromide (25 mg/kg). After a stable airway pressure was established, acetylcholine was injected intravenously (50 μg/kg), and the dynamic airway pressure was measured for 5 min.
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17. Pulmonary eosinophilia was assessed by BAL (*12*).
18. A kidney was excised, and pooled blood was collected for antibody analysis (*12*). Serum was separated by centrifugation and stored at −80°C until analysis. Serum OVA-specific IgE concentrations were determined by sandwich enzyme-linked immunosorbent

Requirement for IL-13 Independently of IL-4 in Experimental Asthma

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The pathogenesis of asthma reflects, in part, the activity of T cell cytokines. Murine models support participation of interleukin-4 (IL-4) and the IL-4 receptor in asthma. Selective neutralization of IL-13, a cytokine related to IL-4 that also binds to the α chain of the IL-4 receptor, ameliorated the asthma phenotype, including airway hyperresponsiveness, eosinophil recruitment, and mucus overproduction. Administration of either IL-13 or IL-4 conferred an asthma-like phenotype to nonimmunized T cell–deficient mice by an IL-4 receptor α chain–dependent pathway. This pathway may underlie the genetic associations of asthma with both the human 5q31 locus and the IL-4 receptor.

Allergic asthma is a complex disorder characterized by local and systemic allergic inflammation and reversible airway obstruction. Asthma symptoms, especially shortness of breath, are primarily related to airway obstruction, and death is almost invariably due to asphyxiation (*1*). Increased airway responsiveness to provocative stimuli, termed airway hyperresponsiveness (AHR), and mucus hypersecretion by goblet cells are two of the principal causes of airway obstruction observed in asthma patients (*2*). Data from animal models consistently reveal a critical role for T_H2 (T helper 2) cells and potentially important roles for the cytokines IL-4 and IL-5 (*3–7*).

T_H2 cells selectively develop and expand in the presence of IL-4 (*8*). To separate direct effects of IL-4 from developmental effects on T_H2 cells in an asthma model, we compared the ability to establish the asthma phenotype in BALB/c mice deficient in either IL-4 or the IL-4 receptor α chain (IL-4Rα) (*9*). After intranasal challenge with the antigen ovalbumin (OVA), BALB/c mice developed a stereotyped asthma phenotype characterized by eosinophil influx of the airways, goblet cell metaplasia with mucus overproduction, and an increase in AHR as revealed by enhanced sensitivity to acetylcholine challenge (*6, 7*). IL-4 and IL-4Rα–deficient mice showed incremental attenuation of each of these asthma indices (Fig. 1, C through E) (*10*). Thus, in agreement with prior studies (*5–7*), IL-4 contributes to the asthma phenotype, but these data suggest an independently greater contribution by IL-4Rα.

IL-13 is a cytokine closely related to IL-4 that binds to IL-4Rα and is also expressed by T_H2 cells from asthma patients (*11*). To assess whether IL-13 might contribute to the asthma

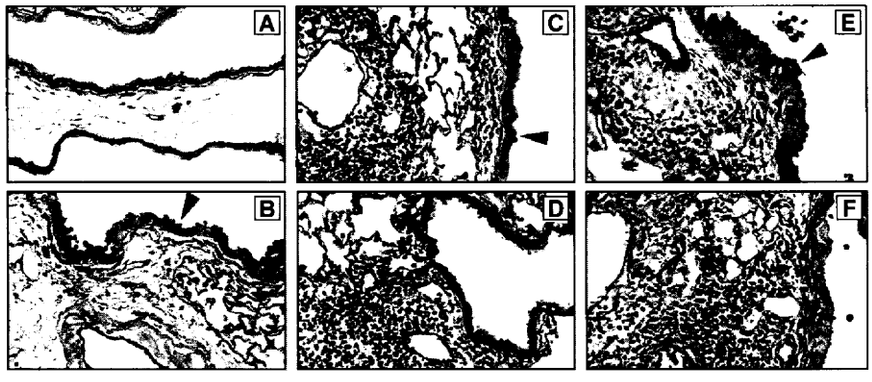

Fig. 1. PAS-stained histologic sections of murine lungs. Arrowheads point to goblet cells within the respiratory epithelium. (**A**) Wild-type mice were primed with OVA and challenged with PBS intranasally. (**B**) Wild-type mice were administered IL-13 intranasally. (**C**) IL-4–deficient and (**D**) IL-4Rα–deficient mice were primed with OVA and challenged with OVA intranasally. Wild-type mice were primed with OVA and challenged intranasally with (**E**) OVA and human Fc control protein or with (**F**) OVA and IL-13R-Fc. Note the marked reduction in goblet cells in (**D**) and (**F**).

assay (ELISA). Sample wells were coated with a 0.01% OVA solution in PBS, blocked with 10% fetal bovine serum (FBS) in PBS, and washed with 0.05% Tween-20 in PBS. Serum samples were diluted 1:10 and 1:100 with 10% FBS in PBS. After an overnight incubation, plates were washed with 0.05% Tween-20 in PBS, and biotin-conjugated anti-mouse IgE (Pharmingen, San Diego, CA) was added. After a wash, avidin peroxidase (0.0025 mg/ml) (Sigma) in 10% FBS/PBS was added, and plates were developed with 2.2′-azino-di(3-ethyl-benzthiazone sulfonate) (Kirkegaard and Perry, Gaithersburg, MD). Plates were read at 405 nm within 30 min. Reported optical density (OD) values are from serum samples that were diluted 1:10, because these values were proven to be below the saturation point of the assay by comparison of OD values of serum samples diluted 1:100 with 10% FBS/PBS.

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