

Blockade of Allergic Airway Inflammation Following Systemic Treatment with a B7-Dendritic Cell (PD-L2) Cross-Linking Human Antibody¹

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We present a novel immunotherapeutic strategy using a human B7-DC cross-linking Ab that prevents lung inflammation, airway obstruction, and hyperreactivity to allergen in a mouse model of allergic inflammatory airway disease. Dendritic cells (DC) have the ability to skew the immune response toward a Th1 or Th2 polarity. The sHlgM12 Ab functions in vitro by cross-linking the costimulatory family molecule B7-DC (PD-L2) on DC up-regulating IL-12 production, homing to lymph nodes, and T cell-activating potential of these APCs. Using chicken OVA as a model Ag, the administration of sHlgM12 Ab to BALB/c mice blocked lung inflammation, airway pathology, and responsiveness to methacholine, even after animals were presensitized and a Th2-polarized immune response was established. This therapeutic strategy was ineffective in STAT4-deficient animals, indicating that IL-12 production is critical in this system. Moreover, the polarity of the immune response upon in vitro restimulation with Ag is changed in wild-type mice, with a resulting decrease in Th2 cytokines IL-4 and IL-5 and an increase in the immunoregulatory cytokine IL-10. These studies demonstrate that the immune response of hypersensitized responders can be modulated using B7-DC cross-linking Abs, preventing allergic airway disease upon re-exposure to allergen. *The Journal of Immunology*, 2004, 173: 1360–1365.

Increase in prevalence, morbidity, and mortality due to asthma in humans over the past two decades has been worldwide (1). The hallmark feature of allergic asthma is abnormal expansion of Th2 cells in the lungs (2, 3). Dendritic cells (DC)⁴ act as the major APCs to naive T cells in lymphoid organs (4), are present in the respiratory tract, and upon isolation from the trachea, bronchi, alveoli, and visceral pleura, are capable of Ag presentation to T cells (5, 6). More importantly, it has been demonstrated using an OVA model of allergic asthma that pulmonary DC prime T cells, inducing a Th2 phenotype. Modulation of DC function to change the polarity of ensuing T cell responses has been a focus of asthma research. GM-CSF and cysteinyl leukotrienes have been used to modulate T cell cytokine polarity in vivo (7–10). In this study, we use an Ab that binds to the costimulatory molecule B7-DC to modify the immune response that underlies inflammatory airway disease in a mouse model of asthma.

We have previously demonstrated the ability of a human IgM mAb designated serum-derived human IgM Ab 12 (sHlgM12) to bind to both human and murine DC in a B7-DC (PD-L2)-dependent manner. This binding resulted in: 1) potentiation of the Ag-

presenting ability of the DC, as seen by the ability to activate OT-I TCR transgenic T cells; 2) increase in survival of the treated DC upon cytokine withdrawal; 3) secretion of IL-12; and 4) homing and/or survival of DC, resulting in increased numbers reaching the draining lymph nodes. The loss in ability by a monomeric form of sHlgM12 Ab to potentiate the immune response, together with the finding that the monomer inhibits the activity of intact pentameric Ab, argues that the targeted determinants on the surface of the DC are activated by cross-linking (11, 12).

B7-DC is one of the more recently identified costimulatory molecules belonging to B7 family. B7-DC is expressed on DC and, upon activation, expressed on macrophages (13, 14). PD-1, the receptor for B7-DC (also known as PD-L2), is expressed on T cells upon activation and acts as a negative regulator as a consequence of an ITIM motif present in its cytoplasmic tail (15). However, this receptor-ligand interaction has been demonstrated to result in both inhibition of certain T cell responses (14) as well as activation of others (13). The dual nature of the responses elicited by B7-DC has been attributed to different kinds of stimulation used to modulate T cell function. However, it is also possible that B7-DC binds to additional receptors that mediate alternative functions. This hypothesis has been advanced recently in studies modeling the structure of the B7 family members and binding studies using T cells from PD-1-deficient mice (16, 17).

In this study, we examine the capability of our newly described B7-DC cross-linking Ab (11, 12) to modulate the immune response in vivo using an OVA model of murine allergic airway inflammation. Because cross-linking B7-DC on DC with sHlgM12 Ab resulted in secretion of IL-12 in vitro, a key cytokine that promotes Th1 responses, we reasoned that this Ab treatment might promote a change in the polarizing influence of DC in vivo, resulting in the protection of mice against allergic airway inflammation, and this protective effect was STAT4 dependent. Indeed, systemic administration of Ab before immunization completely protected mice from symptoms and lesions that mimic allergic

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⁴ Abbreviations used in this paper: DC, dendritic cell; AHR, airway hyperreactivity; BAL, bronchoalveolar lavage; Penh, enhanced pause; pHlgM, polyclonal human IgM; sHlgM12, serum-derived human IgM Ab 12.

airway inflammation. Strikingly, this Ab treatment protected mice from allergic symptoms even when administered 14 days after hypersensitization. Moreover, the polarity of T cells isolated from the spleens of therapeutically treated mice was changed from strong Th2 to weak Th1. We conclude that B7-DC cross-linking Ab treatment protects mice in both a prophylactic and, more importantly, a therapeutic setting in a murine model of allergic airway inflammation.

Materials and Methods

Mice and reagents

Six- to 8-wk-old BALB/c and BALB/c-STAT4^{tm1Gru} mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in the animal house facility, Mayo Clinic, as per the institutional guidelines for further studies. OVA protein was purchased from Sigma-Aldrich (St. Louis, MO). These studies were approved by the Mayo Clinic Institutional Animal Care and Use Committee. The IgM human Ab, sHlgM12, is derived from a patient with Waldenstrom's macroglobulinemia, as described previously (11). Five hundred milligrams of Ab have been purified. The polyclonal human IgM (pHlgM) Ab used as a control in these studies has been described (18).

Immunization and airway challenge

The sensitization and challenge procedure with OVA was modified from the method described by Zhang et al. (19). Briefly, mice were sensitized by an i.p. injection of 100 μ g of OVA adsorbed to 1 mg of alum (Pierce, Rockford, IL). Experimental mice were intranasally challenged with 100 μ g of OVA in PBS under tribromoethanol anesthesia.

Treatment with B7-DC cross-linking Ab

In the prophylactic regimen, mice were treated with sHlgM12 or the control pHlgM Ab i.v. at 10 μ g per day on days -1, 0, and 1 relative to sensitization with OVA in alum. This schedule was designed to determine whether Ab treatment would prevent the establishment of a Th2-polarized response, typically elicited by the use of alum as an adjuvant. In the therapeutic regimen, the Ab treatments were conducted at the same dose and route on the day before the first intranasal challenge, the day of challenge, and day after challenge with OVA in PBS (days 13, 14, and 15 relative to first sensitization with OVA in alum). This treatment schedule was designed to assess whether treatment with B7-DC cross-linking Ab after immunization with a Th2-polarizing regimen could modulate an established response polarity.

Measurement of airway responsiveness to methacholine

Airway responsiveness was assessed on day 27 by methacholine-induced airflow obstruction in conscious mice in a whole body plethysmograph (Buxco Electronics, Troy, NY). Pulmonary airflow obstruction was measured by enhanced pause (Penh) with a transducer connected to preamplifier modules and analyzed by system software. To measure methacholine responsiveness, mice were exposed for 2 min to PBS, followed by incremental dosages of aerosolized methacholine (Sigma-Aldrich). Penh was monitored for each dose.

Collection of bronchoalveolar lavage (BAL) fluid

Immediately after measuring airway hyperreactivity (AHR), animals were injected i.p. with a lethal dose (250 mg/kg) of pentobarbital (Abbott Laboratories, Abbott Park, IL). The trachea was cannulated, and the lungs were lavaged twice with 0.5 ml of HBSS. After centrifugation, the supernatant was collected and stored at -20°C. The cells were resuspended and counted using a hemocytometer. BAL cell differentials were determined with Wright-Giemsa stain; ≥ 200 cells were differentiated using conventional morphologic criteria. IL-5 in the BAL fluid supernatants was measured by ELISA, as directed by the manufacturer (R&D Systems, Minneapolis, MN).

Histology

After BAL fluid collection, the lung was fixed in 10% formalin and embedded in paraffin. Sections were obtained and stained with H&E, and in some cases with anti-CD3 Ab (using a peroxidase-labeled secondary developing reagent). The sections were evaluated by microscopy at $\times 100$ and $\times 400$ magnification.

In vitro cytokine production and proliferation

On day 27, splenocytes from the control Ab or the sHlgM12 Ab-treated mice were harvested and processed. Briefly, after making a single cell

suspension, RBC were lysed by hypertonic shock using ammonium chloride/potassium bicarbonate/EDTA. Cells were counted and resuspended at 3 million cells/ml in RPMI 1640 (Cambrex, Walkersville, MD). OVA was made to a final concentration of 2 mg/ml and was titrated at half log dilutions. Splenocytes were added at 3×10^5 cells in 100 μ l. Supernatants were harvested after 48 h and stored for cytokine assay. Cells were pulsed with [^{3} H]thymidine during the last 18 h of the 72-h assay. Cells were harvested and counted for incorporation of [^{3} H]thymidine (Packard Instrument, Boston, MA).

Stored supernatants were analyzed for IL-4, IL-5, IL-10, IFN- γ , and TNF- α by ELISA, per the manufacturer's protocol (R&D Systems).

Statistical analysis

Data were analyzed using a two-way repeated measures ANOVA or Student's *t* test for normally distributed data and the Whitney rank sum test for nonparametric data.

Results

Prophylactic treatment with sHlgM12 Ab prevents bronchial AHR in murine model of allergic airway inflammation

Treating bone marrow-derived murine DC in vitro with sHlgM12 B7-DC cross-linking Ab resulted in secretion of IL-12 (12). IL-12 is a key cytokine in determining the polarity of the immune response, supporting the Th1 phenotype by promoting the secretion of IFN- γ (20). Skewing the T cell response from a Th2 toward Th1 phenotype can confer protection in allergic disease such as asthma (21). To test whether cross-linking of B7-DC on murine DC with sHlgM12 Ab results in skewing the immune response away from the pathogenic Th2 phenotype induced by immunization with the adjuvant alum, we analyzed the effect of sHlgM12 Ab treatment during the initial immunization with OVA in the induced asthma-like condition in BALB/c mice. Mice were administered sHlgM12 Ab 1 day before, the day of, and 1 day after immunization with OVA in alum (Fig. 1A). This regimen of Ab treatment results in mild reduction in airway responsiveness to methacholine challenge in comparison with the control Ab-treated mice. Mice treated with the sHlgM12 B7-DC cross-linking Ab, however, were significantly protected from airway responsiveness to methacholine challenge relative to animals treated with isotype control Abs (e.g.,

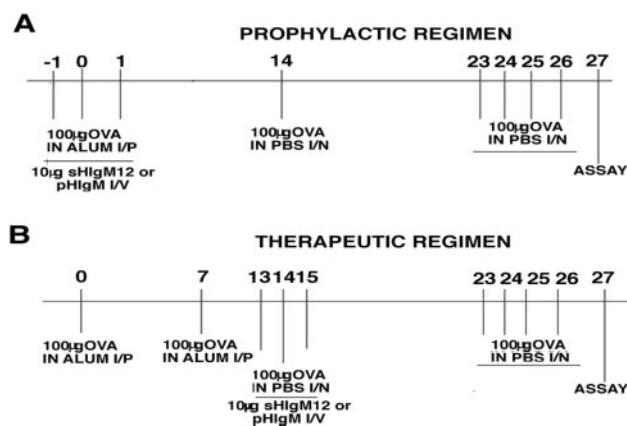


FIGURE 1. Prophylactic and therapeutic Ab treatment schemes. *A*, Prophylactic treatment scheme. Mice received 10 μ g of sHlgM12 or isotype control polyclonal IgM Ab i.v. 1 day before, on the same day, and 1 day after the initial challenge with 100 μ g of chicken OVA with the adjuvant alum. On day 14, animals received their first intranasal challenge with 100 μ g of OVA. Beginning on day 23, animals received repeated intranasal challenges with OVA and were assayed for symptoms of allergic asthma on day 27. *B*, Therapeutic treatment scheme. Animals received two priming treatments with 100 μ g of OVA, one on day 0, and the second on day 7. Treatments with sHlgM12 Ab or polyclonal IgM isotype control Ab began on day 13, and were repeated on days 14 and 15. The intranasal challenges with 100 μ g of OVA were on days 14, 23, 24, 25, and 26.

Fig. 2A, $p = 0.041$). The OVA model of allergic asthma is characterized by pulmonary inflammation reflected by a statistically significant increase in the number of total cells in the BAL, an increase in the number of eosinophils in the BAL, and perivascular and peribronchial cellular infiltrates in lung tissue sections (22). The number of total cells in the BAL fluid was significantly reduced in sHlgM12-treated mice (Fig. 2B, $p = 0.013$). Moreover, sHlgM12 treatment also resulted in prevention of eosinophils migrating to the lungs (Fig. 2C, $p = 0.015$). This failure to detect significant eosinophilic infiltrates correlated with the reduced levels of IL-5 found following treatment with sHlgM12 Ab (Fig. 2D, $p = 0.008$). IL-5 is a cytokine that plays a pivotal role in migration of eosinophils (23). Most striking was the finding that sHlgM12 treatment totally abrogated lung inflammation, the thickening of bronchial epithelium, and the accompanying accumulation of mucus plugs that were readily evident in mice treated with isotype control Abs (Fig. 3, A and B).

Therapeutic treatment with sHlgM12 Ab prevents AHR

Next, we asked whether sHlgM12 Ab treatment could prevent mice from developing allergic airway inflammatory disease in a therapeutic model. In this model, the mice were treated with sHlgM12 Ab on days 13, 14, and 15 following initial sensitization with two priming doses of OVA in alum adjuvant (Fig. 1B). Inflammatory lung disease induced with this regimen was more severe than that induced with a single priming dose of OVA in alum adjuvant. This regimen of Ab treatment provided an opportunity to assess the potential of sHlgM12 Ab to modulate established T cell immune reactivity, in a setting in which the immune response was already skewed toward a pathogenic Th2 polarity. Mice that received the sHlgM12 Ab showed a significant reduction in airway responsiveness to methacholine (Fig. 4A, $p = 0.01$) relative to animals that received isotype control Ab. The responses of sHlgM12-treated animals to methacholine were comparable to the

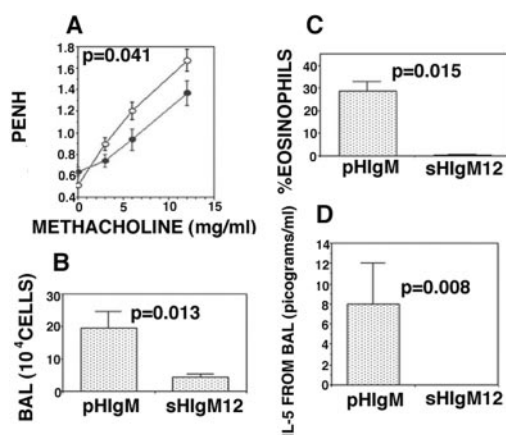


FIGURE 2. Prophylactic effects of sHlgM12 B7-DC cross-linking Ab on OVA-induced AHR and inflammation. **A**, Responsiveness of mice to methacholine challenge. Mice that received either the isotype control polyclonal IgM Ab (○) or the B7-DC cross-linking Ab sHlgM12 (●) were challenged with increasing dosages of methacholine. AHR was measured by Penh, as described in *Materials and Methods*. Data are represented as means $\pm$ SEM ($n = 10$ per group). **B**, Cellular infiltration in lungs. Mice were sacrificed after measuring the AHR to methacholine. Cells present in BAL were counted. Data are represented as means $\pm$ SEM ($n = 10$ /group). **C**, Eosinophilic infiltration in the lungs. The cells from the BAL fluid were stained with Wright-Giemsa for differential counts. Data are represented as the percentage of eosinophils with respect to the total number of cells counted. **D**, IL-5 in the BAL. The amount of IL-5 in the BAL was determined by ELISA. Data are represented as means $\pm$ SEM ($n = 10$ /group).

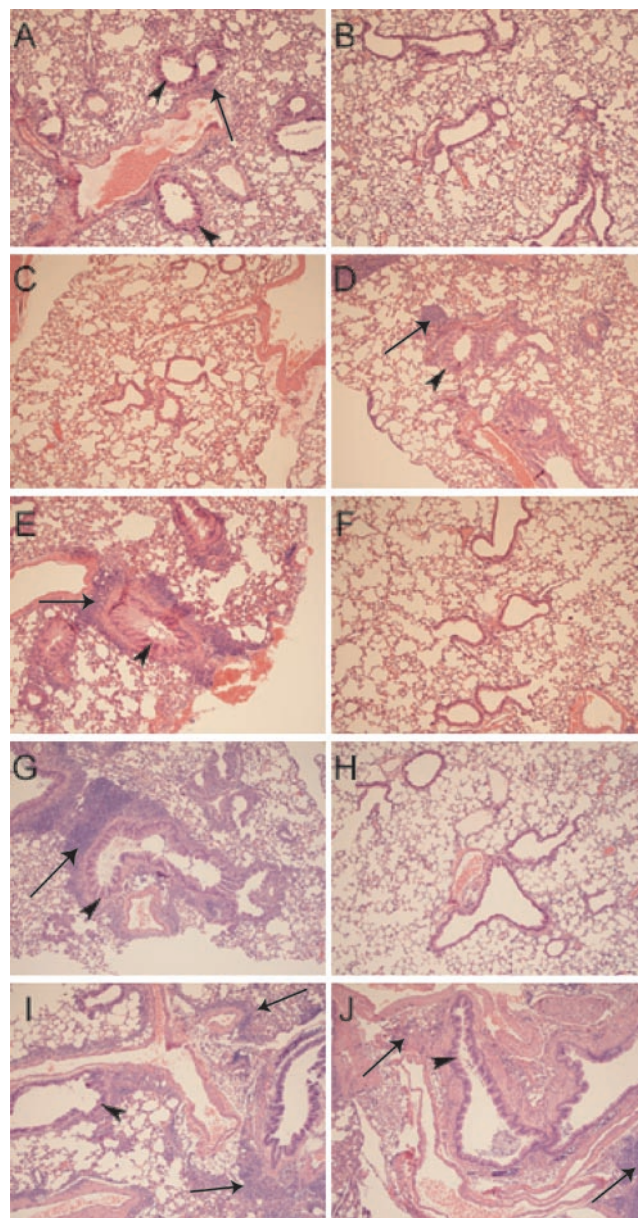


FIGURE 3. Lung pathology. All animals were sensitized with the experimental Ag chicken OVA using either the therapeutic or prophylactic protocols described in Fig. 1, with the exception of the mouse shown in C, which received no treatment. Lungs from all mice were fixed in formalin, and sections were stained with H&E. Data shown are representative of: **A**, BALB/c mice receiving isotype control Ab using prophylactic regimen; **B**, BALB/c mice receiving sHlgM12 B7-DC cross-linking Ab, prophylactic regimen; **C**, naive BALB/c mice; **D**, BALB/c mice receiving PBS during therapeutic regimen; **E**, BALB/c mice receiving isotype control IgM Ab during therapeutic regimen; **F**, BALB/c mice receiving sHlgM12 B7-DC cross-linking Ab during therapeutic regimen; **G**, same as in **E**; **H**, same as in **F**; **I**, STAT4-deficient mice treated with isotype control IgM Ab; and **J**, STAT4-deficient mice treated with sHlgM12 B7-DC cross-linking Ab. Arrows point to areas of inflammation. Arrowhead points to bronchial epithelial thickening caused by airway inflammation. Three to ten animals were studied per group.

responses of untreated, naive animals (Fig. 4A). Moreover, the number of cellular infiltrates in the BAL derived from sHlgM12-treated mice was markedly lower than the infiltrates found in BAL from either the control Ab or the PBS-treated mice (Fig. 4B, $p = 0.008$). The number of cells recovered in the BAL of sHlgM12-treated mice was comparable to the number recovered in normal

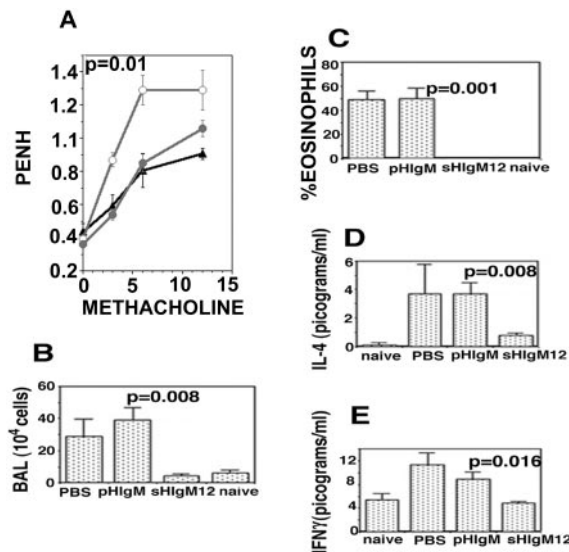


FIGURE 4. Therapeutic treatment of mice with B7-DC cross-linking Ab. *A*, B7-DC cross-linking sHIgM12 Ab treatment 13 days postadministration of Ag abrogates bronchial AHR. Mice that received either the isotype control polyclonal IgM Ab (○) or the sHIgM12 B7-DC cross-linking Ab (●), and untreated, naive mice (▲) were subjected to airway hyperresponsiveness measurement in response to increasing dosage of methacholine, as in Fig. 1. Data are means $\pm$ SEM ($n = 5$ per group). *B*, Absence of cellular infiltration in the BAL. After measuring the AHR, mice were sacrificed, BAL was extracted, and total cells in all the groups were counted. Data are means $\pm$ SEM ($n = 5$ in the Ab-treated groups; $n = 3$ in normal and PBS-treated groups). *C*, Prevention of eosinophilic infiltration in the lungs. The cells from the BAL fluid were stained with Wright-Giemsa for differential counts. There were no detectable eosinophils in either the B7-DC cross-linking Ab-treated group or the normal mice. Data are represented as the percentage of eosinophils with respect to the total number of cells counted. *D* and *E*, Reduced amount of IL-4 and IFN- γ in the lungs of sHIgM12-treated mice. The lungs from all the groups of mice were divided into two halves. One half was homogenized in cold PBS, and the supernatants were subjected to ELISA to measure IL-4 and IFN- γ . Data represent means $\pm$ SEM ($n = 5$ in Ab treatment groups; $n = 3$ in normal or PBS treatment groups).

mice. Similar to our findings with the prophylactic treatment regimen, there was no detectable eosinophilic infiltration in the mice treated therapeutically with sHIgM12 Ab (Fig. 4C, $p = 0.001$).

To further characterize the T cell response pattern, the supernatants from the homogenized lungs of the various groups of mice were analyzed for the prototypic Th1 cytokine, IFN- γ , and the Th2 cytokine, IL-4. The mice that received sHIgM12 showed significantly reduced amounts of both IL-4 (Fig. 4D, $p = 0.008$) and IFN- γ (Fig. 4E, $p = 0.016$) in comparison with the control Ab-treated mice. Although IL-5 levels were not measured in this experiment, the complete absence of eosinophilia suggests that IL-5 levels were most likely low in the sHIgM12-treated animals. This cytokine pattern suggests that sHIgM12 treatment does not lead to a proinflammatory cytokine environment in the lungs by switching the polarity of the T cell response from Th2 to Th1, but rather blocks the development of either kind of T cell response. Immunohistochemistry analysis of lung tissue using CD3-specific Ab as a probe supported this conclusion. Few T cells were present in the lungs of naive animals (Fig. 5A). In contrast, lungs from mice treated with PBS or polyclonal IgM control Ab contained extensive T cell infiltrates (Fig. 5, B and C). Remarkably, the lungs from sHIgM12-treated mice showed no signs of infiltration (Fig. 5D), resembling the lungs of untreated mice (Fig. 5A). In addition, there was no lung pathology in animals that received the therapeutic

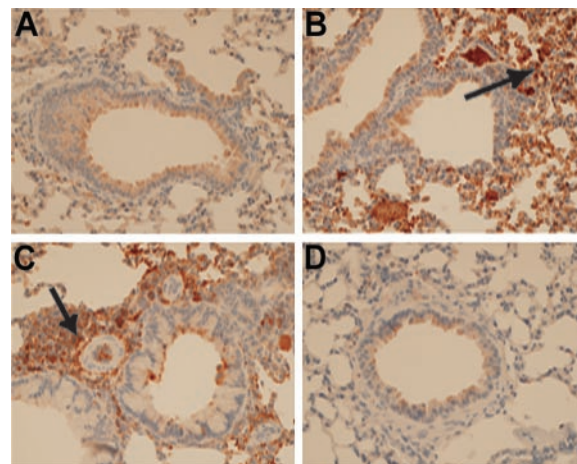


FIGURE 5. Systemic treatment with B7-DC cross-linking Ab blocks T cell inflammation in the lungs. Lungs from naive mice and mice treated therapeutically on days 13, 14, and 15 with PBS; control polyclonal IgM Ab or sHIgM12 B7-DC cross-linking Ab were analyzed for T cell infiltration by histology using rabbit anti-CD3 ϵ and biotin-conjugated goat anti-rabbit/avidin-HRP secondary reagents. Sections were analyzed at $\times 400$. *A*, Represents lungs from naive mice. *B*, Represents lungs from PBS-treated mice. *C*, Represents lungs from control polyclonal IgM Ab-treated mice. *D*, Represents lungs from sHIgM12 B7-DC cross-linking Ab-treated mice. Arrows indicate sites of T cell inflammation marked by staining with anti-CD3 Abs.

treatment protocol of B7-DC cross-linking sHIgM12 Ab 14 days after presensitization; the lungs of naive animals (Fig. 3C) and sHIgM12 Ab-treated mice (Fig. 3F) were indistinguishable. In contrast, animals that were treated with PBS (Fig. 3D) or isotype control Ab (Fig. 3E) exhibited severe distortion of their bronchial airways and substantial inflammatory infiltration.

Our therapeutic strategy was predicated on our early observations that B7-DC cross-linking Ab induces IL-12 production by DC in vitro (12). To determine whether the IL-12 signaling pathways are important for the in vivo therapeutic effects of sHIgM12 Ab treatment, we assessed the ability of Ab to modulate inflammatory airway disease in STAT4-deficient animals. STAT4 is a requisite intermediary that mediates IL-12 signaling (24). STAT4-deficient animals are known to develop highly polarized Th2 responses, as their ability to develop immune responses with Th1 character is severely compromised by the mutation (25, 26). The severity of induced airway inflammatory disease was substantially greater in STAT4-deficient animals (Fig. 3J) relative to wild-type mice (Fig. 3G). Therapeutic treatment of the STAT4-deficient animals with B7-DC cross-linking Ab had no effect (Fig. 3J), while in the same experiment wild-type mice were completely protected from airway inflammatory disease (Fig. 3H). This finding indicates that the ability to activate the STAT4 signaling pathway is critical for the treatment effect of sHIgM12 Ab and provides mechanistic evidence that IL-12 may be important for altering the polarity of the response by presensitized animals.

Treatment with sHIgM12 alters cytokine production in the spleens of allergen-presensitized mice

In the absence of inflammation in the lungs of sHIgM12-treated animals, we examined splenocytes from Ab-treated animals in vitro for the nature of their recall response to OVA challenge to determine whether the Th2 polarity characteristic of an allergic response was altered toward a Th1 polarity. Mice were treated with sHIgM12 Ab or isotype control Ab on days 13, 14, and 15 post-sensitization with OVA in alum adjuvant. The splenocytes were

harvested at day 28 and were restimulated *in vitro* with OVA. The proliferative response of T cells in response to Ag was enhanced 10-fold in mice that had received sHlgM12 treatment in comparison with the control Ab treatment (Fig. 6A). This finding is consistent with our previous observation that treatment of DC with our B7-DC cross-linking Ab enhances their ability to stimulate T cells (11). Furthermore, it demonstrates the potential of this Ab to stimulate cellular responses against isolated proteins, an observation that may have important implications for the development of vaccines. The supernatants from the stimulated cultures were harvested and tested for the presence of cytokines. Although mice that received sHlgM12 produced significantly higher levels of IFN- γ than mice treated with isotype control Ab, neither treatment group produced substantial levels (Fig. 6B, $p = 0.008$). The same trend was observed for the amount of TNF- α produced, in which splenocytes from mice treated with sHlgM12 secrete small amounts of TNF- α , while no TNF- α was detected in control Ab-treated splenocytes (Fig. 6C, $p = 0.029$). In contrast to these Th1 cytokines, the prototypic Th2 cytokines IL-4 and IL-5 were substantially lower in cultures from mice treated with sHlgM12 Ab. Splenic cultures from mice treated with sHlgM12 contained very

small quantities of IL-4 (Fig. 6D, $p = 0.004$) or IL-5 (Fig. 6E, $p = 0.048$). These data indicate that sHlgM12 skews the T cell response toward a Th1 polarity, but that even the Th1 response remains weak, despite a strong proliferative response to secondary Ag challenge. The presence of substantial levels of IL-10 in secondary cultures pretreated with the B7-DC cross-linking Ab sHlgM12 (Fig. 6F, $p = 0.008$) might explain the absence of inflammation in the lungs of mice challenged intranasally with experimental allergen. Despite the ability of T cells in these animals to secrete IFN- γ and TNF- α , T regulatory cells might dampen this tendency and prevent them from tracking to the lungs. Taken together, these data support the notion that sHlgM12 treatment protects presensitized individuals from allergic airway inflammatory disease by the ability of the Ab to prevent a Th2 type of environment and also due to its ability to promote the secretion of the anti-inflammatory cytokine, IL-10.

Discussion

The incidence and severity of allergic airway inflammation are increasing nationally (27). The general improvement in hygiene during the last century may be responsible as the immune system seems to be conditioned by childhood infections. The hypothesis is that the absence of infection during childhood leads to a predisposition later in life to develop allergic responses to inhaled environmental Ags (28). We recently described a novel approach for the potentiation of the immune response to Ag using a human mAb that binds to mouse and human DC (11). This treatment differs from conventional methods (treatment with CD40L, LPS, or CpG-oligodeoxynucleotides) used to activate DCs in that maturation phenotypes indicated by up-regulation of CD80, CD86, and MHC molecules are not induced. Animals treated with conventional DC activators experience splenomegaly, while mice treated with sHlgM12 Ab do not. Treatment of DCs with sHlgM12 Ab *in vitro* induces IL-12 and alters the migration pattern of DCs transplanted into mice (12). Remarkably, systemic treatment of mice with very small quantities (30 μ g) of Ab can induce the same migration of DC to draining lymph nodes and potentiate Ag presentation of Ag-pulsed transplanted DC that reach the nodes (12).

The mechanism of DC activation by sHlgM12 Ab is not fully understood. We have shown that Ab binding to DC is dependent on expression of the costimulatory molecule B7-DC, and that B7-DC-binding ligands and B7-DC-specific IgG Abs can partially block the binding of the human mAb. Because monomers of the pentameric IgM Ab fail to activate, and in fact block, the activation by the native Ab, we have concluded that the cross-linking ability of the Ab is a critical feature of this reagent. We are now acquiring data (not described in this work) demonstrating conclusively that treatment of cultured DCs (both human and mouse) with sHlgM12 Ab activates specific signaling pathways and reproducibly up-regulates subsets of genes. These new findings tend to support the hypothesis that the systemic immunologic consequences of treatment with sHlgM12 are related to Ab-induced changes in resident DCs. Another, nonexclusive possibility is that Ab administered *in vivo* interferes with the interaction of B7-DC with a natural ligand. One such ligand is PD-1, a known negative regulator of T cell function (29). The possibility that additional undefined ligands exist is also plausible. Just how disruption of interactions between B7-DC and its ligands *in vivo* might influence the course of immunity is not known at present.

One possible explanation of the therapeutic effect of sHlgM12 is that B7-DC cross-linking by sHlgM12 Ab might lead to development of new sets of T cells that are skewed to secrete IFN- γ in response to IL-12 and IFN- γ secreted by the DC. This new lineage of T cells might then suppress the development of effector Th2

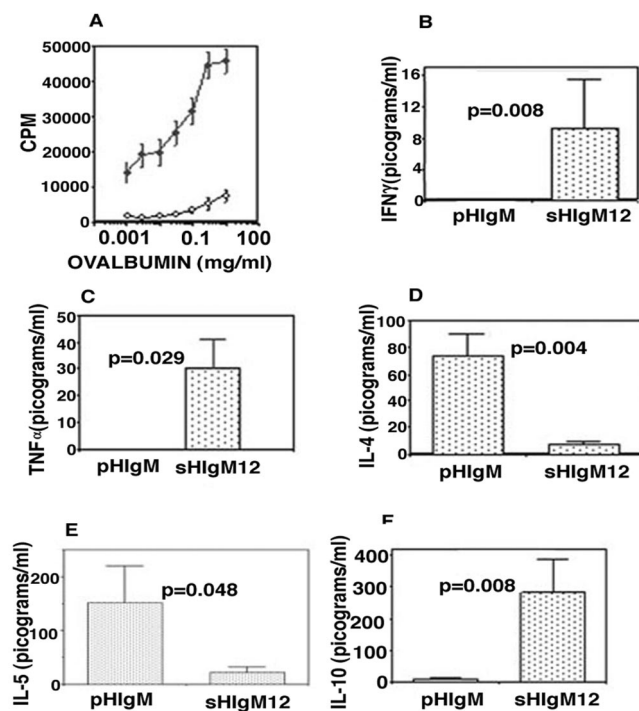


FIGURE 6. Treatment of mice with B7-DC cross-linking Ab postadministration of Ag results in reduced IL-4 and IL-5 secretion and increased IFN- γ , TNF- α , and IL-10 in T cells upon *in vitro* recall. **A**, Splenocytes from mice that received B7-DC cross-linking Ab display stronger proliferative responses to secondary OVA challenge. Splenocytes were harvested from the sHlgM12 and pHlgM Ab-treated mice and stimulated *in vitro* with titrating amounts of OVA protein in triplicates. Cells were pulsed with [^{3} H]thymidine after 3 days. The open symbols represent the response of the control Ab-treated group, while the filled symbols represent the response of the B7-DC cross-linking Ab-treated group. Data represent the mean $\pm$ SEM ($n = 5$). **B–F**, Cross-linking B7-DC results in secretion of Th1 type of cytokines IFN- γ and TNF- α , reduced secretion of Th2-type cytokines IL-4 and IL-5, and increased levels of IL-10. The splenocytes were harvested from the two groups of mice and were restimulated *in vitro* with OVA. The supernatants were collected after 72 h and were tested for IFN- γ and TNF- α by ELISA. Data represent mean $\pm$ SEM ($n = 5$). T cells from naive mice or mice treated with PBS Ab failed to incorporate substantial [^{3} H]thymidine (<300 cpm) or to spontaneously secrete IFN- γ (<1 pg/ml).

cells from the memory Th2 cell population, and, hence, IL-4 and IL-5 secretion in the vicinity of the lungs (30). The finding that STAT4-deficient mice are not responsive to sHlgM12 Ab treatment is consistent with this hypothesis. However, the possibility that STAT4 is an important signaling intermediary for a B7-DC ligand also remains open to question.

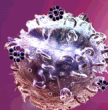
A recent report documents the ability of bone marrow-derived DC to secrete IFN- γ in response to IL-12 and skew the T cell response toward a Th1 polarity (31). However, we have only detected very small increases in IFN- γ production in these mice. Spleen cells from Ab-treated mice display a profoundly altered cytokine secretion pattern, suggesting that systemic Ab treatment alters the outcome of subsequent intranasal Ag challenge. Remarkably, no inflammation in the lungs occurs. The up-regulation of IL-10 production by splenocytes suggests the possibility that systemic treatment with sHlgM12 Ab may induce immunomodulatory regulatory T cells that suppress the effector responses in the lungs. A recent report demonstrates the ability of DCs to stimulate T regulatory cells in a costimulation-dependent fashion (32). The details of this kind of response still need to be established.

The possible involvement of PD-1, an inhibitory receptor for B7-DC (PD-L2) expressed by activated T cells, in immunomodulatory effects of sHlgM12 Ab treatment is not known. It remains possible that administration of the IgM Ab blocks interactions between DC and T cells, altering the course of T cell activation. However, it should be noted that very small quantities of Ab are administered during this treatment regimen and the affinity of the Ab for B7-DC is low. In previous experiments, DC treated in vitro with sHlgM12 Ab, and washed, retained the biological effects of treatment even when no Ab was detectable by flow cytometry on their cell surfaces (11). Therefore, we favor the hypothesis that the Ab acts by altering DC function, rather than by blocking interactions between DC and T cells. The view that the Ab acts directly on DC is supported by the finding that sHlgM12 Ab alters the cytokine secretion profile of DC in vitro (12) (S. Radhakrishnan and L. R. Pease, unpublished observations). In a very recent study, i.p. administration of an IgG Ab, specific for B7-DC and F(ab')₂ of that Ab, exacerbates airway inflammatory disease, presumably by blocking B7-DC interactions with PD-1 (33). This finding highlights the unique therapeutic value of our systemic treatment protocol using an IgM B7-DC cross-linking Ab.

An important feature of this model is the human origin of the sHlgM12 Ab. Importantly, the Ab binds to human DCs as well as to mouse DCs, and induces comparable intracellular molecular changes in both species in vitro (S. Radhakrishnan and L. R. Pease, unpublished observations). Although it remains to be seen whether systemic treatment of human patients with this Ab can alter the course of asthmatic responses, this is an exciting possibility.

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Retraction: Blockade of Allergic Airway Inflammation Following Systemic Treatment with a B7-Dendritic Cell (PD-L2) Cross-Linking Human Antibody

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Letter of Retraction

We wish to retract the article titled “Blockade of Allergic Airway Inflammation Following Systemic Treatment with a B7-Dendritic Cell (PD-L2) Cross-Linking Human Antibody,” by Suresh Radhakrishnan, Koji Iijima, Takao Kobayashi, Moses Rodriguez, Hirohito Kita, and Larry R. Pease, *The Journal of Immunology*, 2004, 173: 1360–1365.

In the course of investigating suspicious patterns of experimental results in the laboratory, a systematic and in-depth study of key findings in this article was carried out using blinded protocols. In these repeat studies, no evidence was found to support our original conclusions that B7-DC XAb modulates dendritic cell functions. We do not believe our failure to reproduce our earlier findings is the result of a technical problem. A member of the B7-DC XAb investigative team, Dr. Suresh Radhakrishnan, who was involved in or had access to all the work on this subject, was found in a formal investigation to have engaged in scientific misconduct in unpublished experiments involving the B7-DC XAb reagent. This finding of misconduct and our inability to reproduce key findings using blinded protocols has undermined our confidence in our published report. We seek, therefore, to retract this body of work.

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