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Costimulation Blockade Inhibits Allergic Sensitization but Does Not Affect Established Allergy in a Murine Model of Grass Pollen Allergy[1]

Birgit Linhart,* Sinda Bigenzahn,† Arnulf Hartl,‡ Christian Lupinek,* Josef Thalhamer,§ Rudolf Valenta,[2]* and Thomas Wekerle[2,3]*†

Type I allergy is characterized by the development of an initial Th2-dependent allergen-specific IgE response, which is boosted upon a subsequent allergen encounter. Although the immediate symptoms of allergy are mainly IgE-mediated, allergen-specific T cell responses contribute to the late phase as well as to the chronic manifestations of allergy. This study investigates the potential of costimulation blockade with CTLA4Ig and an anti-CD154 mAb for modifying the allergic immune response to the major timothy grass pollen allergen Phl p 5 in a mouse model. BALB/c mice were treated with the costimulation blockers at the time of primary sensitization to the Phl p 5 allergen or at the time of a secondary allergen challenge. Costimulation blockade (CTLA4Ig plus anti-CD154 or anti-CD154 alone) at the time of sensitization prevented the development of allergen-specific IgE, IgM, IgG, and IgA responses compared with untreated but sensitized mice. However, costimulation blockade had no influence on established IgE responses in sensitized mice. Immediate-type reactions as analyzed by a rat basophil leukemia cell mediator release assay were only suppressed by early treatment but not by a costimulation blockade after sensitization. CTLA4Ig given alone failed to suppress both the primary and the secondary allergen-specific Ab responses. Allergen-specific T cell activation was suppressed in mice by early as well as by a late costimulation blockade, suggesting that IgE responses in sensitized mice are independent of T cell help. Our results indicate that T cell suppression alone without active immune regulation or a shifting of the Th2/Th1 balance is not sufficient for the treatment of established IgE responses in an allergy. ***The Journal of Immunology,*** **2007, 178: 3924–3931.**

Immunoglobulin E-mediated allergy affects >25% of the population worldwide with a continuously increasing prevalence (1). Several studies suggest that the induction of IgE responses leading to allergic sensitization occurs very early in childhood, perhaps even in pregnancy upon allergen encounter (2). Almost all important allergens are protein/peptide Ags that, after presentation to the immune system of atopic individuals under certain conditions (e.g., environmental factors, Ag dose, or mode of Ag encounter) preferentially activate Th2 cells to produce cytokines such as IL-4 and IL-13 (3–5). These cytokines, together with CD40/CD154 (CD40L) interactions, are required for the class switch recombination of B cells to IgE (6). This process results in the formation of allergen-specific IgE Abs that bind to FcεRs on a variety of inflammatory cells and, after repeated allergen contact, mediate the immediate and late symptoms of allergic disease (7, 8). After the primary sensitization event has taken place, repeated encounter with allergens leads to a strong increase of systemic allergen-specific IgE Ab levels and IgE-mediated symptoms (9). The continuous boosting of secondary IgE responses may be one of the important mechanisms that promotes the transition from mild (e.g., rhinoconjunctivitis) to severe allergic manifestations (e.g., asthma) (10).

Th2 cells play a central role in the initial allergic sensitization and in the chronic inflammatory cascades of allergic diseases. Several approaches for the suppression or modulation of T cell activity have therefore been developed to prevent and treat allergy. They include the induction of T cell tolerance with allergen-derived T cell epitope-containing peptides, the use of IL-4 antagonists, treatment with anti-CD4 Abs or immunosuppressive agents such as cyclosporine, and experimental strategies aimed at the induction of tolerance via the activation of tolerogenic APCs or T regulatory cells (4, 11, 12).

Another possible option for the prevention and treatment of allergic immune responses may be interference at the level of costimulation (13). In this work we studied the effect of costimulation blockade on the immune responses to the major timothy grass (*Phleum pratense*) pollen allergen Phl p 5 in a murine model of grass pollen allergy (14). CTLA4Ig, a soluble fusion protein consisting of the extracellular domain of CTLA4 linked to the IgG_1 Fc region, is known to block the CD28-B7 pathway (15). Anti-CD154 (CD40L) mAbs interfere with the CD154-CD40 pathway, mainly by blocking the engagement of CD40 (16) but potentially also through other mechanisms (17). CTLA4Ig and anti-CD154 Abs have been shown to be powerful costimulatory blocking reagents in numerous models of transplantation and autoimmunity (13).

*Christian Doppler Laboratory for Allergy Research, Division of Immunopathology, Department of Pathophysiology, Center of Physiology and Pathophysiology and †Division of Transplantation, Department of Surgery, Medical University of Vienna, Austria; ‡Department of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria; and §Department of Molecular Biology, University of Salzburg, Salzburg, Austria

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[2] R.V. and T.W. are cosenior authors of this article.

[3] Address correspondence and reprint requests to Dr. Thomas Wekerle, Medical University of Vienna, Division of Transplantation, Department of Surgery, Vienna General Hospital, Waehringer Guertel 18-20, A-1090 Vienna, Austria. E-mail address: thomas.wekerle@meduniwien.ac.at

Table I. *Immunization and treatment protocol*[a]

Group	Sensitization	Treatment		
	rPhl p 5	Anti-CD154	CTLA4Ig	Early/Late
A	+			
B	+	Days 0, 2, and 4		Early
C	+		Days 0, 2, and 4	Early
D	+	Days 0, 2, and 4	Days 0, 2, and 4	Early
E	+	Days 21, 23, and 25		Late
F	+		Days 21, 23, and 25	Late
G	+	Days 21, 23, and 25	Days 21, 23, and 25	Late

[a] Groups of six BALB/c mice (A–G) were sensitized with an aluminum hydroxide-adsorbed recombinant timothy grass pollen allergen, Ph1 p 5, on days 0 and 21. Treatment with anti-CD154 and/or CTLA4Ig was given at the time of sensitization (groups B–D, "early," on days 0, 2, and 4) or second immunization (groups E–G, "late," on days 21, 23, and 25) as shown in the table.

CTLA4Ig (abatacept) has recently been approved for the treatment of rheumatoid arthritis (18), and a second generation version (LEA29Y (belatacept)) is currently being evaluated in renal transplantation (19). Little is known, however, about the potential effect of these costimulation blockers on allergic immune responses; in particular, no studies dissecting the humoral response to defined clinically relevant allergens have been reported. Our results demonstrate that costimulation is essential for the establishment of an allergen-specific immune response at the time of allergic sensitization. In contrast, costimulation blockade did not affect an already established IgE Ab response. These findings have important implications for the development of therapeutic strategies for allergic diseases.

Materials and Methods

Recombinant allergens and antibodies

Purified recombinant timothy grass pollen allergens (rPhl p 2 and rPhl p 5) (20) were obtained from Biomay. The hamster anti-mouse CD154 (MR1) Ab was purchased from BioExpress. Human CTLA4Ig was generously provided by Bristol-Myers Squibb. Both reagents have been used to successfully block T cell responses in several mouse models of transplantation (21–24). For control experiments, purified human IgG_1 and hamster IgG were obtained from Sigma-Aldrich and ICN Biomedicals, respectively.

Animals

Female 5–8 wk old BALB/c and C57BL/6 (B6) mice were purchased from Charles River Laboratories and kept under specific pathogen-free conditions. All experiments were approved by the local review board of the Medical University of Vienna and were performed in accordance with national and international guidelines of laboratory animal care.

Immunization and treatment of BALB/c mice

Groups of mice ($n = 6$) were sensitized s.c. (day 0 and day 21) with 5 μg of rPhl p 5, adsorbed to $Al(OH)_3$ (Alu-Gel-S; SERVA Electrophoresis) (14). Treatment (anti-CD154 mAb; human CTLA4Ig) (0.5 mg/mouse) was given i.p. on days 0, 2, and 4 (designated as "early") or on days 21, 23, and 25 (designated as "late"). Intraperitoneal treatment with corresponding amounts of hamster IgG or human IgG_1 was performed for control purposes. The sensitization and treatment schedules for each group of mice are displayed in Table I. Blood samples were taken from the tail veins and serum was stored at −20°C until analysis.

B cell epitope mapping using recombinant allergen fragments

λgt11 clones expressing B cell epitopes of the major rye grass pollen allergens Lol p 5A and Lol p 5B and empty wild-type phage were probed for reactivity with mouse IgG_1 Abs induced by sensitization to Phl p 5 as described (14, 25). *Escherichia coli* Y1090 was grown overnight in Luria Bertani medium containing 0.4% (w/v) maltose and 50 μg/ml ampicillin, harvested by centrifugation, and resuspended in 10 mM $MgSO_4$. Cells were dissolved in 0.6% (w/v) agarose and plated onto Luria-Bertani plates containing 50 mg/L ampicillin. Two-microliter aliquots of phage lysates containing $>10^5$ PFU were dotted onto the plates. Plates were incubated at 43°C until plaques became visible and protein synthesis was induced by overlay with nitrocellulose filters (Schleicher & Schuell) soaked with 10 mM isopropyl β-D-thiogalactoside for 4 h at 37°C. Filters were cut into stripes. The stripes, containing the expressed allergen fragments from clones 11, 14, 21, 26, 47, 50, 57, 59, 68, 81, 117, and 120 and the phage λgt11 as negative control or 1 μg of rPhl p 5 as positive control, were incubated with mouse sera diluted 1/1000 overnight, a monoclonal rat anti-mouse IgG_1 Ab (BD Pharmingen) diluted 1/1000 for 5 h, and a ^{125}I-labeled goat anti-rat IgG Ab (Sigma-Aldrich) diluted 1/2000 for 2 h. Reactivity with the allergen fragments was detected by autoradiography. The intensities of the signals were determined by densitometry using the AlphaEaseFC ChemiImager 4400 software.

ELISA experiments

To measure Ag-specific Abs in the sera of immunized mice, an ELISA was performed as described earlier (14, 26). Plates were coated with rPhl p 5 (5 μg/ml), sera were diluted 1/10 for IgE, 1/100 for IgM, IgA, and IgG2a, and 1/1000 for IgG1, and bound Abs where detected with monoclonal rat anti-mouse IgM, IgG_1, IgE, IgA, and IgG_{2A} Abs (BD Pharmingen) diluted 1/1000 and a HRP-coupled goat anti-rat antiserum (Amersham Biosciences) diluted 1/2000.

T cell proliferation assay

Spleens were removed under aseptic conditions (day 100) and homogenized. After the lysis of erythrocytes, cells were washed and resuspended in complete medium (RPMI 1640, 10% FCS, 0.1 mg/ml gentamicin, and 2 mM glutamine). Single cell suspensions were plated into 96-well round-bottom plates at a concentration of 2×10^5 cells/well (200 μl) in triplicates and stimulated with or without Con A (0.5 μg/well), rPhl p 2 (3 μg/well), and rPhl p 5 (3 μg/well) for 4 days. The cultures were pulsed with 0.5 μCi/well tritiated thymidine for 16 h and harvested. The proliferation responses were measured by scintillation counting. The ratio of the mean proliferation after Ag stimulation and medium control values, i.e., the stimulation index (SI),[4] was calculated.

Rat basophil leukemia (RBL) assay

For the quantification of IgE Ab-mediated, immediate-type reactions, RBL cell mediator release assays were performed as previously described (27). RBL-2H3 cells were cultivated in 96-well tissue culture plates (4×10^4 cells/well) for 24 h at 37°C using 7% CO_2. Passive sensitization was performed by incubation with 1/30 diluted murine sera for 2 h. Cells were washed twice with Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.4 mM NaH_2PO_4, 5.6 mM D-glucose, 12 mM $NaHCO_3$, 10 mM HEPES, and 0.1% w/v BSA (pH 7.2)) to remove unbound Abs. Degranulation of RBL cells was induced by adding 0.3 μg/ml rPhl p 5. After 30 min, β-hexosaminidase release was analyzed. Results are expressed as percentages of total β-hexosaminidase released after the addition of 1% Triton X-100 and represent the mean of triplicate determinations.

Mixed lymphocyte reaction

As a positive control for the biological activity of CTLA4Ig, an MLR was performed in the presence of 100 μg/ml CTLA4Ig. Spleen cells were

[4] Abbreviations used in this paper: SI, stimulation index; RBL, rat basophil leukemia.

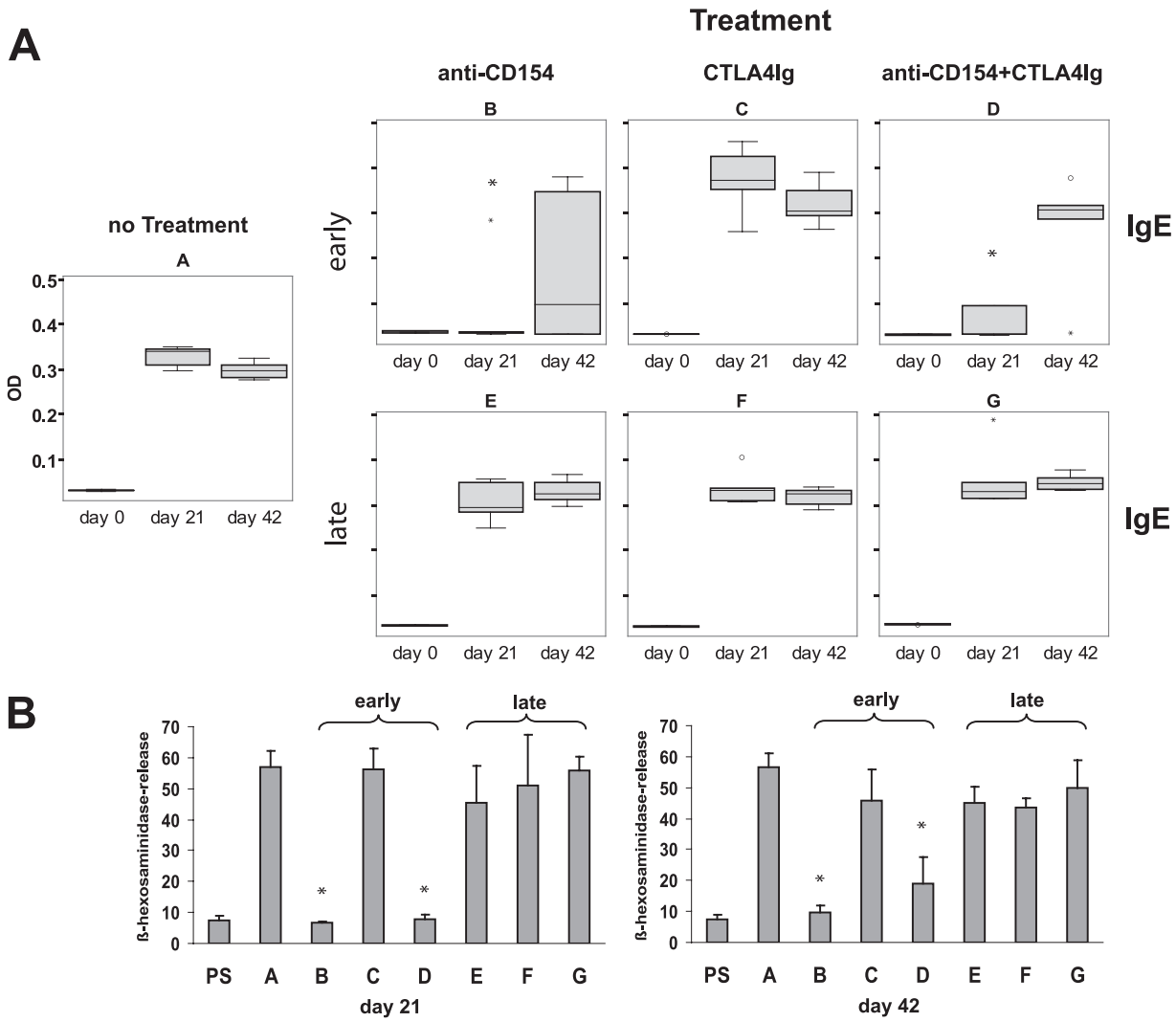

FIGURE 1. Costimulation blockade with CTLA4Ig plus anti-CD154 inhibits allergic sensitization but does not affect an already established allergic secondary IgE response. *A*, Serum levels of rPhl p 5-specific IgE measured by ELISA on days 0, 21, and 42 are shown in box-and-whiskers plots. *B*, The biological activity of allergen-specific IgE was studied by RBL degranulation assays. RBL cells were loaded with serum IgE of Phl p 5-sensitized mice (groups A–G) obtained on day 21 and 42 or with the corresponding preimmune sera (PS) and challenged with the allergen. The mean β–hexosaminidase release ± SD of each group of mice is shown on the *y*-axis. *, $p < 0.05$; indicates a significant reduction compared with the control, group A.

washed with PBS and resuspended in MLR medium (42.5 ml of RPMI 1640 (BioWhittaker), 7.5 ml of controlled processed serum replacement type 2 (CPSR-2; Sigma-Aldrich), 0.5 ml of HEPES buffer (ICN Biomedicals), 1.55 ml of nutrient mixture (10 ml of distilled water, 5 ml of nonessential amino acids, 0.055 g of sodium pyruvate, 0.146 g of L-glutamine, 5000 U of penicillin, and 5000 μg of streptomycin), and 100 μl of 2-ME (dilution 1/3000)). B6 responder cells (4×10^5) were incubated at 37°C and 5% CO_2 with 4×10^5 irradiated (30 Gy) stimulator cells of either BALB/c or B6 mice or with medium in triplicates. After 3 or 4 days, cells were pulsed with [^{3}H]thymidine and incubated for 12–18 h. After harvesting on filter plates, the cells were analyzed with a MicroBeta 1450 beta counter (Wallac Products; HVD Life Science). SI values were calculated by dividing the mean counts per minute from responses against self (B6), and against the allogeneic stimulator (BALB/c) by mean background counts per minute (i.e., counts per minute with no stimulator population).

Statistical analysis

Ab levels are illustrated by box-and-whisker plots. Differences between groups B–G and the control, group A, were assessed with a Wilcoxon-Mann-Whitney *U* test and exact significances were determined and adjusted using the Bonferroni-Holm method. SPSS statistical software system 14.0 was used for calculations. The reported *p* values are results of two-sided tests. $p \leq 0.05$ was considered statistically significant.

Results

Establishment of a murine model for grass pollen allergy by sensitization of mice with the timothy grass pollen allergen Phl p 5

Mice sensitized with Phl p 5 developed an IgE Ab response specific for Phl p 5 already at day 21 (Fig. 1*A*, *group A*). Passive transfer of serum IgE from the sensitized mice but not of their preimmune serum led to the sensitization of RBL cells and the release of β-hexosaminidase after challenge with Phl p 5 (Fig. 1 *B*, *group A*). Sensitized mice developed also Phl p 5-specific IgG_1 Abs that were detectable at day 21 and continued to increase after repeated immunization (Fig. 2, *group A*). Phl p 5-sensitized mice cross-reacted with group 5 allergens from other grass species, including Lol p 5 from rye grass (*Lolium perenne*) (data not shown). We could therefore analyze the epitope spectrum recognized by sensitized mice using phage clones expressing recombinant fragments of the two isoforms (Lol p 5A and Lol p 5B) of the rye grass allergen Lol p 5, which had been identified with IgE Abs from grass pollen allergic

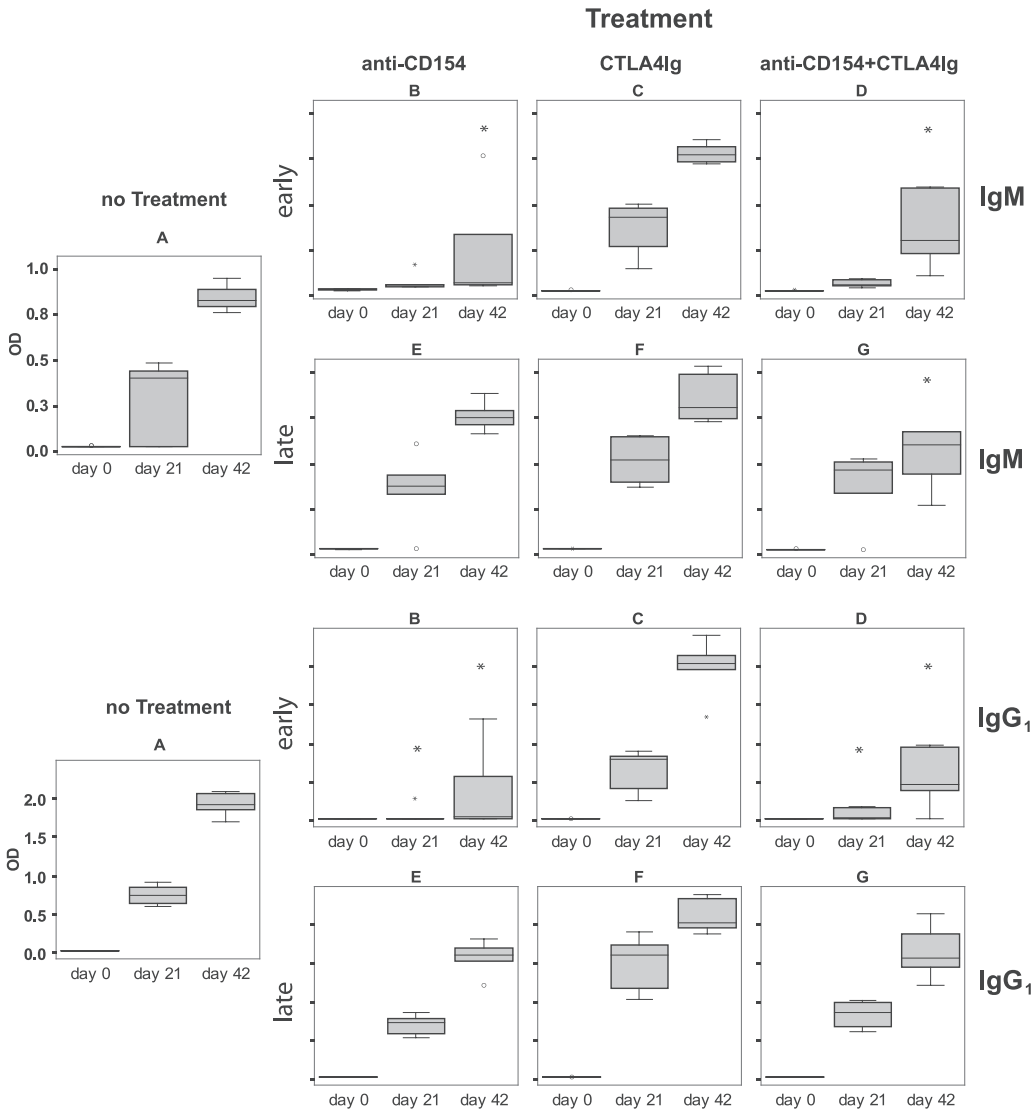

FIGURE 2. Early treatment with CTLA4Ig plus anti-CD154 but not late treatment prevents the induction of IgM and IgG1 Ab responses to the major timothy grass pollen allergen Phl p 5. Serum samples, collected on days 0, 21, and 42 after primary immunization were analyzed for Phl p 5-specific IgM and IgG_1 Abs by ELISA. Ab levels are displayed as box-and-whiskers plots, *, $p < 0.05$; indicates a significant reduction compared with the untreated but sensitized group A.

patients (Fig. 3). As determined by densitometry, >75% (average 78%) of Phl p 5-specific Abs bound to the Lol p 5-derived fragments. Most of the sensitized mice developed Ab responses against the clone 81-defined Lol p 5B fragment (Fig. 3), which corresponds to an N-terminal fragment of Phl p 5 (timothy grass, *P. pratense*) and is highly homologous to group 5 allergens from other grasses and group 6 allergens from timothy grass. The clone 81-defined fragment corresponds to a highly allergenic domain of Phl p 5 that is recognized by most grass pollen allergic patients (25), underscoring the clinical relevance of our model.

Costimulation blockade inhibits allergic sensitization

The effects of costimulation blockade on allergen-specific primary immune responses (i.e., allergic sensitization) and on the secondary allergen-specific immune responses (i.e., boosting of an allergen-specific immune response) was investigated by the administration of costimulation blockers CTLA4Ig and/or an anti-CD154 mAb at the time of sensitization or at the time of the secondary allergen challenge (i.e., 3 wk after sensitization) (for group descriptions see Table I).

First, we investigated the effect of costimulation blockade on allergic sensitization in our murine allergy model ("early" treatment). In BALB/c mice that had been sensitized to the major grass pollen allergen Phl p 5 (group A), allergen-specific IgE could be detected 3 wk after sensitization by ELISA. Almost no Phl p 5-specific IgE responses could be detected in groups B and D, which had received treatment with anti-CD154 or anti-CD154 plus CTLA4Ig at the time of sensitization. The effect of early costimulatory blockade at days 0, 2, and 4 was limited, because it was possible 3 wk later to sensitize mice with a second injection of Phl p 5. This second immunization induced an allergen-specific IgE Ab response in groups B and D similar to that observed for the untreated group after primary immunization (Fig. 1*A*). The inhibition of allergic sensitization seemed to depend mainly on the anti-CD154 Ab treatment, because the application of this Ab alone (group B) yielded a similar result as that of treatment with both Abs (group D). This assumption is supported by the finding that treatment with

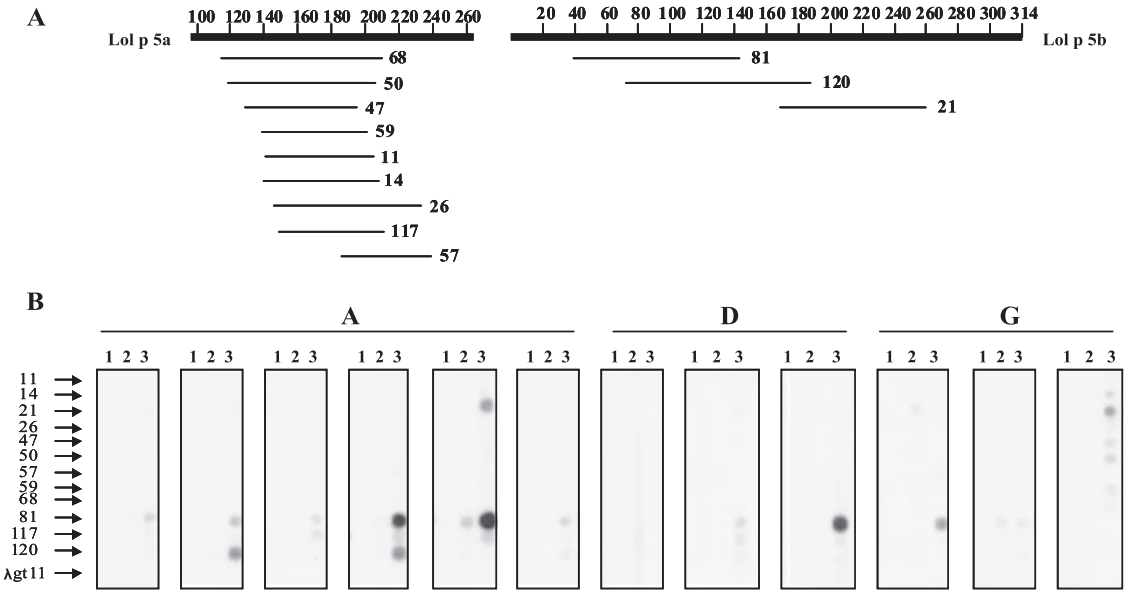

FIGURE 3. Epitope-mapping of the Phl p 5-specific IgG_1 response in immunized mice. *A*, Schematic representation of Lol p 5A and Lol p 5B fragments expressed by the phage clones. *B*, Lol p 5A- and Lol p 5B-derived clones (11–120) and a phage clone without an insert (λgt11) were immobilized to a nitrocellulose filter in the order given and probed with mouse sera from groups A, D, and G obtained before sensitization (*lanes 1*), on day 21 (*lanes 2*), and on day 42 (*lanes 3*).

CTLA4Ig alone (group C) did not suppress allergic sensitization to rPhl p 5 (Fig. 1*A*). Despite treatment with CTLA4Ig the induction of a Phl p 5-specific IgE response was comparable to that of the untreated, sensitized control group A, although the activity of the CTLA4Ig fusion protein had been confirmed in a MLR using the same batch of protein (data not shown).

The impact of costimulation blockade on IgE AB-mediated immediate allergic reactions was also demonstrated using RBL cell degranulation assays. When mouse sera of the sensitized but untreated group A were loaded on RBL cells, a strong degranulation of basophils upon allergen challenge (57% β-hexosaminidase-release 3 and 6 wk after sensitization) was observed, confirming the presence of allergen-specific IgE in the serum (Fig. 1*B*). By contrast, sera collected at day 21 from groups B and D, which had received costimulation blockade at the time of sensitization, failed to elicit degranulation. The data obtained with the RBL assay were thus in agreement with the measured levels of IgE in the serum. Administration of CTLA4Ig at the time of sensitization failed to prevent allergic sensitization. Furthermore, mice (groups B and D) that had been sensitized with a second injection of Phl p 5 21 days after the costimulation blockade exhibited a β-hexosaminidase release of 10 and 19%, respectively, after 6 wk (Fig. 1*B*).

Costimulation blockade has no influence on a secondary IgE response

To analyze the effect of costimulation blockade on an already established allergic immune response (i.e., secondary immune response or boosting of an established response), 3 wk after sensitization the groups of mice that had been sensitized to rPhl p 5 received treatment with anti-CD154 alone (group E), CTLA4Ig alone (group F), or a combination of anti-CD154 plus CTLA4Ig (group G) (Table I). In Fig. 1*A* the "late" row shows the development of Phl p 5-specific IgE Ab titers in these groups 3 and 6 wk after sensitization. On day 21 after the first allergen contact, mice of groups E, F, and G had developed Phl p 5-specific IgE Abs comparable to those of the sensitized and untreated controls of group A. Upon secondary allergen contact (i.e., second injection of Phl p 5 at day 21), the IgE Ab levels in the treated groups E, F, and G were almost identical with those in the untreated sensitized control group A, indicating that the IgE response to Phl p 5 was not influenced by costimulation blockade after sensitization. We again determined the allergenic activity of these Phl p 5-specific IgE Abs in an RBL degranulation assay, confirming that there was no effect of late costimulation blockade on allergic immune responses because there was no significant difference in the β-hexosaminidase releases from rat basophils between the untreated group A and mice of groups E, F, and G (Fig. 1*B*).

Costimulation blockade prevents the initiation of allergen-specific IgM, IgG, and IgA responses but does not affect allergen-specific secondary Ab responses

BALB/c mice, which had been sensitized with the major grass pollen allergen Phl p 5 on days 0 and 21, had Phl p 5-specific IgM, IgG_1, IgG_{2A}, and IgA Ab responses already at day 21 (Figs. 2 and 4). Treatment with the anti-CD154 Ab alone and with the combination of both costimulation blockers given at the time of sensitization blocked the development of Phl p 5-specific IgM as well as the IgG_1, IgG_{2A}, and IgA responses. As observed for IgE, CTLA4Ig alone was not able to suppress allergen-specific Ab responses. The suppressive effect of costimulation blockade on humoral responses had disappeared at day 21, because the second injection of Phl p 5 at day 21 could induce Phl p 5-specific Ab responses in mice of groups B and D (Figs. 2 and 4).

Similarly as observed for IgE, costimulation blockade given after the development of an allergen-specific Ab response did not suppress allergen-specific IgM, IgG, and IgA secondary immune responses in mice of groups E, F, and G (Figs. 2 and 4). Animals from groups E, F, and G had already developed a Phl p 5-specific Ab response comparable to that of the untreated but sensitized control group A. This allergen-specific Ab response could be boosted upon subsequent immunization with Phl p 5, which resulted in an increase of allergen-specific Ab levels 6 wk after primary immunization (Figs. 2 and 4). Thus, no strong effect of costimulation blockade on secondary Ab responses became apparent.

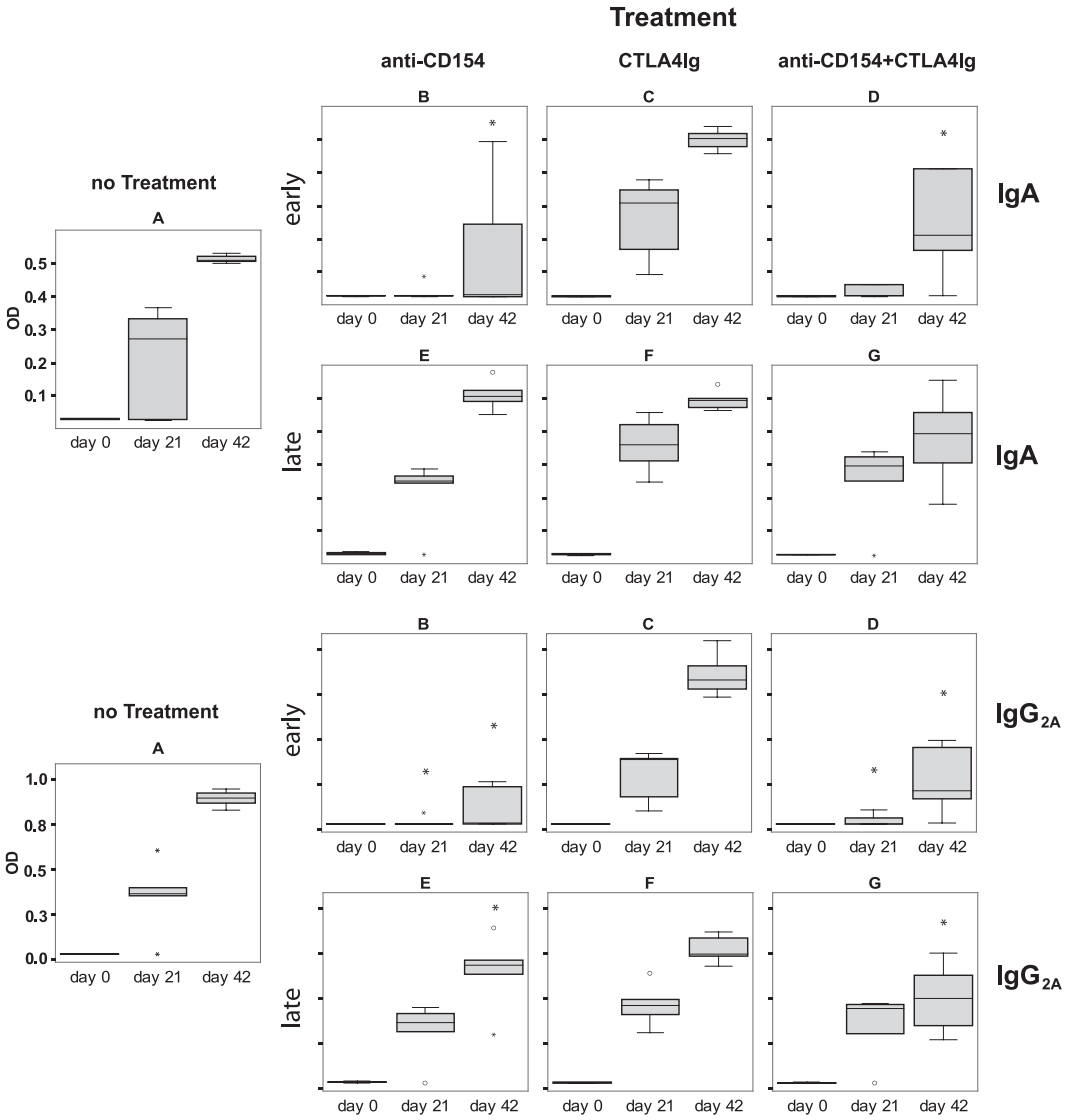

FIGURE 4. Effects of costimulation blockade on rPhl p 5-specific IgA and IgG_{2A} Ab responses. Serum samples, collected on days 0, 21, and 42 from the mice of groups A–G were analyzed for Phl p 5-specific IgA and IgG_{2A} Abs by ELISA. Ab levels (OD values) are displayed as box-and-whiskers plots, *, $p < 0.05$; indicates a significant reduction compared with the untreated but sensitized group A.

Possible subtle differences between the Ab levels on day 42 were not analyzed in detail. Mice that were not sensitized to Phl p 5 but had been treated with CTLA4Ig and anti-CD154 or isotype control Abs did not mount any detectable humoral response to the Phl p 5 allergen (data not shown).

Costimulation blockade induces allergen-specific nonresponsiveness of T cells

We further investigated whether spleen-derived T cells isolated from the various groups of mice were able to proliferate upon stimulation with rPhl p 5 (Fig. 5). Spleen cells from untreated Phl p 5-sensitized mice strongly proliferated in response to Phl p 5 (mean SI,: 18.5). Phl p 5-specific proliferation of splenocytes was substantially inhibited in treated groups B, D, E, and G, resulting in mean stimulation indices between 1.5 and 2. In our model, CTLA4Ig treatment alone (groups C and F) was not sufficient for the complete suppression of allergen-specific T cell responses, although T cell responses were considerably reduced (mean SI: 2.6 and 3.6). Spleen cells of mice from the various groups did not proliferate in response to an immunologically unrelated allergen, Phl p 2 (20) (Fig. 5).

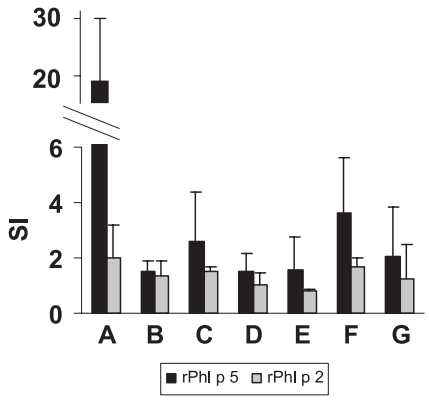

FIGURE 5. Treatment with CTLA4Ig and/or anti-CD154 reduces lymphoproliferative responses to Phl p 5. T cell proliferation was measured at day 100 in spleen cell cultures after in vitro stimulation with timothy grass pollen allergens Phl p 5 (filled bars) or Phl p 2, an immunologically unrelated grass pollen allergen (negative control) (gray bars). The bars represent the mean stimulation indices (SI ± SD) for the different groups of mice.

Discussion

Sensitization, i.e., the induction of allergen-specific IgE Abs, is the initial event in the establishment of allergic disease. Subsequent allergen contact leads to a boost of allergen-specific IgE and T cell responses as well as to allergic inflammation (28). Allergic inflammation can be elicited via IgE-mediated activation of different inflammatory cells but may also occur via IgE-independent T cell activation (11, 28).

In this study, we investigated the effects of costimulation blockade on allergic sensitization and allergen-specific secondary IgE responses. The CTLA4Ig and anti-CD154 mAb that we used for suppression of T cell help have previously been described as potent costimulatory blockade reagents and were effective in numerous models of transplantation and autoimmunity, but also in clinical trials for rheumatoid arthritis and psoriasis (18, 21–23, 29). When these reagents were given in combination in the course of allergic sensitization, they efficiently prevented the induction of a primary IgE response to the major timothy grass pollen allergen Phl p 5. This result confirms the pivotal role of the CD40-CD40L interaction (30–32) in the induction of an allergen-specific IgE response and also in T cell-dependent B cell responses in general, as the induction of an IgM and IgG response to rPhl p 5 was also inhibited. CTLA4Ig, when given alone at the time of sensitization with an allergen, did not effectively prevent a primary IgE response while dampening allergen-specific T cell reactivity to some degree. Several studies have suggested that CD28 blockade through CTLA4Ig can have distinct effects on Th1 vs Th2 responses, but results differ depending on the specific experimental system investigated (13). In several models Th2 responses were less susceptible to CTLA4Ig treatment (33–35), which is compatible with our observation that in the presented allergy model a Th2-driven humoral response was not blocked by CTLA4Ig.

Interestingly, CTLA4Ig and anti-CD154 failed to inhibit secondary IgE responses and IgE-mediated allergen-specific degranulation of basophils in mice with an already established allergy even though T cell responses had been strongly suppressed (as revealed by the results of the proliferation assays). Our data thus suggest that established secondary IgE responses are difficult to control via T cell-based therapeutic approaches. In fact, it is well established that the priming of naive T cells during sensitization is crucial to provide help for B cell differentiation, but the role of T cells in the secondary immune responses of memory B cells is a matter of controversy (36–40). Previous studies in mice by using T cell depleting anti-CD4 Abs suggested that memory B cell persistence needs little or no Ag-mediated T cell help (36). Also, the influence of T cells on the activation of memory B cells has recently been investigated. Using a model of T and B cell deficient $RAG^{-/-}$ mice for adoptive transfer experiments, the activation of virus-specific memory B cells to secrete IgG was shown to be independent of cognate or bystander T cell help (37).

The lack of effect of costimulation blockade on secondary IgE responses and IgE-mediated allergic responses in already sensitized mice does not contradict earlier studies showing that costimulation blockade can suppress T cell-mediated allergic inflammation, because several reports have provided convincing evidence for the coexistence of IgE-mediated as well as IgE-independent, T cell-mediated reactions in allergic patients (4, 11). This may also explain why attempts to treat allergic symptoms by down-regulating allergen-specific T cell responses in asthmatic patients using allergen-derived T cell peptides led to controversial results (41, 42).

In fact, the blockade of the CD28/CTLA4-CD80/CD86 pathway could block allergen-induced peripheral blood T cell proliferation as well as the IL-5 production of asthmatic subjects (43) and abrogate airway hyperresponsiveness in a murine model of allergic asthma (44). Furthermore, it has been demonstrated that the aerosol-induced effector functions of memory T cells could be inhibited by blocking the function of the B7-1 or B7-2 ligands (45–47). As a consequence, the blockade of costimulation at the time of allergen exposure has been proposed as a potential strategy for the treatment of atopic disease (48).

Costimulation blockers exert their effects through several mechanisms. Both anti-CD154 and CTLA4Ig have been shown to be able to induce anergy, deletion, and regulation (24, 49). The contribution of each of these mechanisms in vivo depends on the specifics of the model used (13). It is likely that regulation was induced by anti-CD154 mAb and CTLA4Ig in the present study but, if so, it was not sufficient to suppress the secondary IgE response.

We think that the differential effects of costimulation blockade on allergic inflammation can be explained by the existence of at least two pathomechanisms, one involving IgE-mediated allergic inflammation that seems to be less susceptible to T cell-mediated control, and the other involving non-IgE-mediated but T cell-dependent allergic inflammation, a mechanism that can be controlled by T cell epitope-derived peptides, anti-CD4 Abs, cyclosporine, and perhaps regulatory T cells (4, 11). In addition, there may be a link between the latter two mechanisms because it has been shown that T cell activation in allergic patients can be regulated by IgE-facilitated Ag presentation (8).

Our data therefore suggest that it will be difficult to treat all facets of established allergic disease using strategies focusing only on the induction of T cell tolerance. It will rather be necessary to regulate established allergic immune responses by using strategies that actively antagonize the established immune response (e.g., regulatory immune responses, shifting the balance toward Th1, or the induction of counterimmune responses) (11, 50). The dissection of the various pathways and their importance for allergic inflammation as well as the determination of their sensitivity for various immunological treatment strategies should conceivably result in the selection of optimal immunological strategies for the treatment of allergic diseases.

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