

Blocking IL-15 Prevents the Induction of Allergen-Specific T Cells and Allergic Inflammation In Vivo

René Rückert,* Katja Brandt,* Armin Braun,[†] Heinz-Gerd Hoymann,[†] Udo Herz,[‡] Vadim Budagian,* Horst Dürkop,[§] Harald Renz,[‡] and Silvia Bulfone-Paus^{1*}

IL-15 has been shown to accelerate and boost allergic sensitization in mice. Using a murine model of allergic sensitization to OVA, we present evidence that blocking endogenous IL-15 during the sensitization phase using a soluble IL-15R α (sIL-15R α) suppresses the induction of Ag-specific, Th2-differentiated T cells. This significantly reduces the production of OVA-specific IgE and IgG and prevents the induction of a pulmonary inflammation. Release of proinflammatory TNF- α , IL-1 β , IL-6, and IL-12 as well as that of Th2 cytokines IL-4, IL-5, and IL-13 into the bronchi are significantly reduced, resulting in suppressed recruitment of eosinophils and lymphocytes after allergen challenge. It is of clinical relevance that the airway hyper-responsiveness, a major symptom of human asthma bronchiale, is significantly reduced by sIL-15R α treatment. Ex vivo analysis of the draining lymph nodes revealed reduced numbers of CD8, but not CD4, memory cells and the inability of T cells of sIL-15R α -treated mice to proliferate and to produce Th2 cytokines after in vitro OVA restimulation. This phenomenon is not mediated by enhanced numbers of CD4⁺/CD25⁺ T cells. These results show that IL-15 is important for the induction of allergen-specific, Th2-differentiated T cells and induction of allergic inflammation in vivo. *The Journal of Immunology*, 2005, 174: 5507–5515.

During an immune response, naive CD4⁺ or CD8⁺ T cells are primed to Ags by dendritic cells (DCs),² expand, and acquire effector functions. Cytokines present during the initial priming determine whether a type 1 or type 2 effector T cell will develop. Influenced by the surrounding cytokine network and by signals from APCs, T cells synthesize cytokines that further support the initiated immune response (1). IL-4 and IL-5, cytokines produced by both CD4⁺ and CD8⁺ T cells, are implicated in the induction as well as the maintenance of allergic sensitization and inflammation. Besides the long-accepted role CD4⁺ T cells play during these allergic processes, it becomes more and more evident that CD8⁺ T cells are also important for the induction of allergic sensitization and atopic diseases, which has been shown in different models (2). Interestingly, in a murine model of allergic sensitization to OVA, the development of airway hyper-reactivity, eosinophil influx, and IL-5 production seems to be correlated directly to CD8⁺ T cells (3). After the acute immune response has subsided, an increased frequency of Ag-specific memory T cells persists for years after the initial priming. This generation and maintenance of CD8⁺ memory cells is dependent on the cytokine IL-15, which selectively promotes the survival of CD44/CD122⁺ memory phenotype cells (4–6). In addition, IL-15 is a cytokine

that promotes activation and proliferation of T cells as well as their migration (7) and cytokine release (8). We have previously shown that IL-15 inhibits apoptosis of T and B cells (9) as well as epithelial cells (10, 11) and that exogenous IL-15 potently stimulates Ab production in a murine Th2 model of allergic sensitization (12). The latter designates IL-15R-mediated signaling as a potentially interesting target for the suppression of immune responses leading to allergic diseases.

Because IL-15 is also produced by DCs (13) and epithelial cells (11), it influences the local activation and differentiation as well as the migration of T cells to the site of inflammation (14). Interaction of T cells with antigenic peptides on the surface of IL-15-producing APCs (15) and appropriate costimulatory signals provided by these APCs result in a series of events culminating in T cell activation. Generation of DCs in the presence of IL-15 boosts their potential to activate T cells and to induce T cell-mediated immune responses in vivo (16, 17). DC-derived IL-15 is able to induce the proliferation of naive and memory CD8⁺ T cells (6) and to enhance the differentiation of Th2 cells when given together with IL-4 (18).

Blocking endogenous IL-15 in vivo by a soluble IL-15R α (sIL-15R α) as antagonist has previously been shown to be a very efficient treatment in inflammatory, collagen-induced arthritis, where Ag-induced immunopathology, including production of anti-collagen Abs, was significantly reduced, but the mechanisms and the cells involved were not investigated in detail (19). Because we had demonstrated previously that IL-15 accelerates and enhances Ab production during allergic sensitization (12) and promotes the induction of CD8⁺-mediated immune responses, we tested the ability of sIL-15R α in inhibiting allergic sensitization to OVA and pulmonary inflammation in a mouse model of allergic asthma bronchiale (20).

We present the first evidence that blocking IL-15 abrogates induction of Ag-specific, Th2 T cells, preventing the induction of an allergic bronchial inflammation, recruitment of inflammatory cells, and, therefore, the induction of airway hyper-reactivity.

*Department of Immunology and Cell Biology, Research Center Borstel, Borstel, Germany; [†]Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany; [‡]Department of Pathobiochemistry and Laboratory Medicine, University of Marburg, Marburg, Germany; and [§]Department of Pathology, Universitäts Klinikum Benjamin Franklin, Berlin, Germany

Received for publication August 25, 2004. Accepted for publication February 11, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Address correspondence and reprint requests to Dr. Silvia Bulfone-Paus, Department of Immunology and Cell Biology, Research Center Borstel, Parkallee 22, 23845 Borstel, Germany. E-mail address: sbulfone@fz-borstel.de

² Abbreviations used in this paper: DC, dendritic cell; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; DLN, draining lymph node; MCh, methacholine chloride; R_L, pulmonary resistance; sIL-15R α , soluble IL-15R α .

Materials and Methods

Treatment

BALB/c mice were analyzed in three groups and treated as shown in Fig. 1A. Control mice were treated with diluent (PBS) and Imject alum adjuvant (1.5 mg Al(OH)₃; Pierce) on days 1, 7, and 14 only. In the OVA group, mice were sensitized i.p. as previously described (20) with 10 µg of OVA (grade VI; Sigma-Aldrich) adsorbed to 1.5 mg of Al(OH)₃ on day 1, 7, and 14. In the OVA plus sIL-15Rα group, in addition to the OVA sensitization, mice were treated i.p. with sIL-15Rα (50 µg in 200 µl of PBS) from days 1 to 10 and on days 15 and 16. To induce a pulmonary, allergic inflammation, mice of all groups were challenged inhalationally with OVA aerosol on days 15 and 16 (1% solution in PBS, 15 min nebulized with Pari Master; Pari). Blood was collected from the tail vein on days 10 and 16 for measurement of specific Ab titers. On day 17, mice were killed, a bronchoalveolar lavage (BAL) was performed, and spleens and draining lymph nodes (DLN) were excised. All animal studies have been approved by institutional and governmental review boards. The sIL-15Rα was produced in *Escherichia coli* according to the method of Ruchatz et al. (19) and was used previously in a Th1 model (6). The sIL-15Rα activity was tested by inhibiting IL-15 (but not IL-2)-induced proliferation of the IL-2/IL-15-dependent T cell line CTLL-2 during 48-h culture, as assessed by [³H]thymidine incorporation (Fig. 1B). Endotoxin was removed from the sIL-15Rα preparation using polymyxin (Sigma-Aldrich); endotoxin contamination was excluded using a *Limulus* assay. Treatment with sIL-15Rα in vivo was nontoxic, because mice showed no neurological abnormalities and had normal fur appearance. Also, the cellular composition of spleen and lymph nodes as well as the number of apoptotic cells were not changed by sIL-15Rα treatment. The concentration of 50 µg of IL-15Rα/mouse/day has been systematically tested and successfully used by our group in previous studies, where it has been shown to be efficient in the suppression of overwhelming immune responses (6). To allow meaningful comparisons with our previous work, it was advised to continue using this established concentration. This was encouraged and justified by the fact that similar sIL-15Rα concentrations have been shown to be efficient in several different published in vivo models; e.g., blocking of cardiac allograft rejection (21) has been achieved by injecting 60 µg of sIL-15Rα daily for 10 days, and prevention of collagen-induced arthritis was successful after injection of 40 µg of sIL-15Rα daily for 14 days (19).

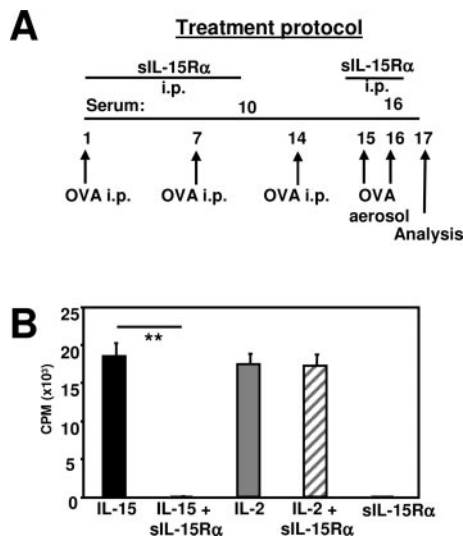


FIGURE 1. Treatment. **A**, BALB/c mice were treated and analyzed in three groups. Control mice were treated with diluent only (sterile PBS). OVA mice were sensitized i.p. with OVA on days 1, 7, and 14 and challenged with OVA aerosol on days 15 and 16. OVA + sIL-15Rα mice were OVA sensitized and treated additionally i.p. with sIL-15Rα on days 1–10 and during the challenge on days 15 and 16. Blood was collected on days 10 and 16 for determination of serum Ab titers. On day 17, mice were killed, and either BAL was performed, or lungs were removed for histological analysis. **B**, The high sIL-15Rα level significantly inhibits IL-15-induced, but not IL-2 induced, proliferation of the CTLL-2 T cell line; sIL-15Rα alone did not induce significant proliferation.

Cell culture and cytokine detection

Single cell suspensions were prepared on day 17 and incubated at 1×10^6 cells/ml for 5 days with medium (RPMI 1640, 10% inactivated FCS, 5 mmol/L glutamine, 100 µg/ml streptomycin, and 100 U/ml penicillin) or stimulated with OVA (25 µg/ml; grade VI) or OVA plus sIL-15Rα (1 µg/ml) in the presence of IL-2 or IL-15 (10 ng/ml; Tebu). Cell culture supernatant or cell-free BAL fluids were analyzed for cytokine production using a Bio-Plex kit (Bio-Rad). Fifty microliters per sample was analyzed on the Luminex 100 (Bio-Rad) according to the manufacturer's instructions.

FACS analysis

Cells were analyzed by FACS using Abs against CD4, CD8, CD44, CD122, or CD25 (all from BD Pharmingen). For CFSE analysis, lymph node cells were labeled with CFSE (Molecular Probes) according to the manufacturer's instructions (with 5 µM CFSE in PBS), stimulated for 72 h with OVA (10 µg/ml), and double stained with CD8⁺ or CD4-allophycocyanin and CD44-PE. Propidium iodide (Sigma-Aldrich) was added to exclude dead cells.

Proliferation assay

Single cell suspensions of spleen cells and DLN were prepared on day 17 and stimulated with OVA (25 µg/ml), OVA plus IL-2, or OVA plus IL-15 (10 ng/ml) for 48 h and labeled for 20 h with 0.2 µCi of [³H]thymidine. Con A (3 µg/ml) was used as a positive control for maximal activation.

Ig ELISA

Determination of serum OVA-specific Ab titers was performed by ELISA as previously described (20). Allergen-specific Ab titers of the samples were related to a standard pool serum generated from hyperimmunized BALB/c mice and deliberately assigned to be 2000 U/ml (20). The detection limit was 1 U.

Bronchoalveolar lavage

BAL was prepared on day 17 as previously described (20). Briefly, mice were killed, and the lungs were rinsed twice with 0.7 ml of cold PBS. BAL fluid was centrifuged; cells were counted and used for cytopins, which were stained with Papanheim. Cells were differentiated following standard morphological criteria. Cytokines were analyzed in cell-free BAL fluid.

Histology

Lungs were inflated in situ with 1 ml of 4% Formalin, excised, fixed in formalin, embedded in paraffin, cut, and stained with Giemsa.

Detection of airway hyperresponsiveness (AHR)

On day 17, mice were anesthetized with i.p. injections of etomidate (total dose, 21–38 mg/kg) and fentanyl (0.05–0.10 mg/kg) with minimal supplementations as required. AHR was then assessed after challenge with aerosolized methacholine chloride (MCh; Sigma-Aldrich) in orotracheally intubated, spontaneously breathing mice as described previously (22).

Table I. Primer sequences^a

Primer Sequences
IL-4 sense 5'-GCTAGTTGTCATCCTGCTC-3'
IL-4 antisense 5'-GTGATGTGGACTTGGACTC-3'
IL-9 sense 5'-ATGTTGGTGACATACATCCTTGC-3'
IL-9 antisense 5'-CGGCTTTTCTGCCTTTGCATCTC-3'
IL-13 sense 5'-GACTGCAGTCTTGCTCTTGC-3'
IL-13 antisense 5'-TGAGTCCACAGCTGAGATGCC-3'
IFN-γ sense 5'-CCTCAGACTCTTTGAAGTCT-3'
IFN-γ antisense 5'-CAGCGACTCCTTTTCTCTT-3'
IL-12p40 sense 5'-CTCACCTGTGACACGCCTGA-3'
IL-12p40 antisense 5'-CAGGACATCAATACTCTCTC-3'
SOCS2 sense 5'-GGGAGCTCTTTACCAACA-3'
SOCS2 antisense 5'-AAAGGCCCATTTGATCTTGA-3'
SOCS3 sense 5'-TGCAAGGGGAATCTTCAAC-3'
SOCS3 antisense 5'-AGCTCACCAGCCTCATCTGT-3'
β-Actin sense 5'-GTGGGGCGCCCGAGGCACCA-3'
β-actin antisense 5'-CTCTTAATGTCACGCACGATTTTC-3'

^a SOCS2, Suppressor of cytokine signaling.

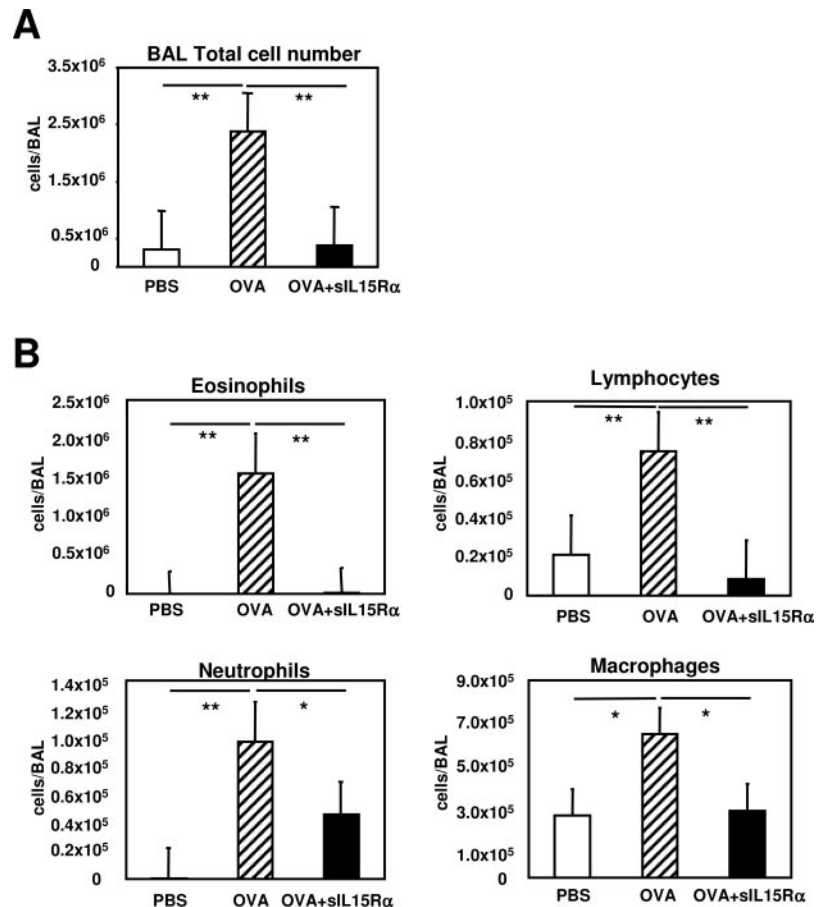


FIGURE 2. Treatment with sIL-15R α prevents allergic infiltration of leukocytes into the airways. BAL was performed on day 17. *A*, OVA- plus sIL-15R α -treated mice (■) had an absolute BAL cell number comparable to that of controls (□), representing a highly significant reduction compared with OVA alone (▨, **, $p \leq 0.001$). *B*, Differential cell counts of BAL performed on cytopins revealed the complete absence of an allergic inflammation in OVA-sensitized, sIL-15R α -treated mice. Significance was determined using Student's *t* test, and data are shown as the mean $\pm$ SD of eight to 10 mice per group.

Briefly, mice were placed in a supine position in a body plethysmograph, and pulmonary resistance (R_L) was calculated from the measured signals of transpulmonary pressure and tidal respiratory flow and was continuously recorded using commercial software (HEM 3.4; Notocord). After recording of baseline values, AHR to aerosolized MCh in dose steps of 0.125, 0.25, 0.5, and 1 μ g was assessed from the increase in R_L . Dried aerosols were generated by a computer-controlled, jet-driven aerosol generator system (particle size, 2.1 μ m; Bronchy III, MMAD; Fraunhofer ITEM, licensed by Buxco). Aerosol concentrations were determined by a gravimetrically calibrated photometer. The total dose inhaled via the orotracheal tube in micrograms was calculated and controlled by a computerized dose control system (Fraunhofer ITEM) based on the continuously measured respiratory minute volume and aerosol concentration. From dose-effect plots (maximum percent change in R_L vs MCh dose) the effective inhalation dose in micrograms of MCh needed to produce a 100% increase in resistance was evaluated (ED_{100}) for each animal.

RT-PCR

RNA was extracted from 5×10^6 LN cells, isolated on day 17, and stimulated for 24 h with OVA (10 μ g/ml). cDNA was synthesized using random hexanucleotide primers and SuperScript Preamplification System II (Invitrogen Life Technologies) and was amplified using 1 U of AmpliTaq DNA polymerase (Roche). The final primer concentration was 0.5 μ M. Cycling conditions were 5 min at 94°C and an additional 30 s at 94°C. The annealing temperature was 60°C for 30 s, followed by 30 s at 72°C. Thirty cycles were performed, with a final extension at 72°C for 10 min. Primers (Table I) were obtained from Metabion. To exclude contamination, all experiments were run with a mock PCR. β -Actin amplification was used to normalize the cDNA amount.

Statistical analysis

Results are presented as the mean $\pm$ SD from pooled data of at least three identical experiments, all of which gave comparable results ($n = 9$ –17 mice/group). Student's *t* test for unpaired samples was used to determine statistical differences. If not otherwise stated, all differences between PBS (controls) and OVA groups were significant.

Results

Blocking IL-15 inhibits allergic inflammation

IL-15 activates mast cells and eosinophilic granulocytes as well as T cells, and boosts and accelerates allergic sensitization. Thus, to analyze the role of IL-15 in detail and to evaluate a possible therapeutic use of IL-15 antagonists, we blocked IL-15 activities during the allergic sensitization using a sIL-15R α in a mouse model of allergic sensitization to OVA.

After i.p. OVA sensitization, mice were exposed to OVA aerosol on days 15 and 16. Histological analysis and BAL were performed on day 17 (see Fig. 1*A* for details). As shown in Fig. 2*A*, OVA- plus sIL-15R α -treated mice show an absolute cell number in the BAL comparable to that in untreated control mice, which represents a highly significant reduction compared with OVA alone ($p \leq 0.001$). Differential cell counts of the BAL revealed the

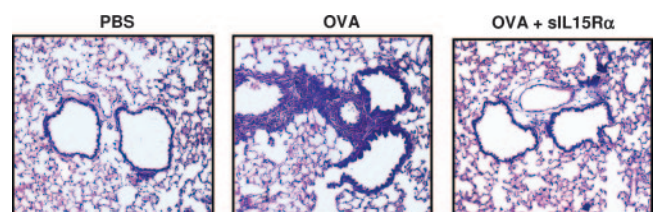


FIGURE 3. Soluble IL-15R α blocks the induction of allergic inflammation in mice. Lungs were isolated on day 17, fixed in formalin, embedded in paraffin, cut, and stained with Giemsa. OVA-sensitized mice developed a strong peribronchial infiltrate containing numerous eosinophilic granulocytes after OVA challenge. Treatment with sIL-15R α completely prevented the development of such pulmonary inflammation.

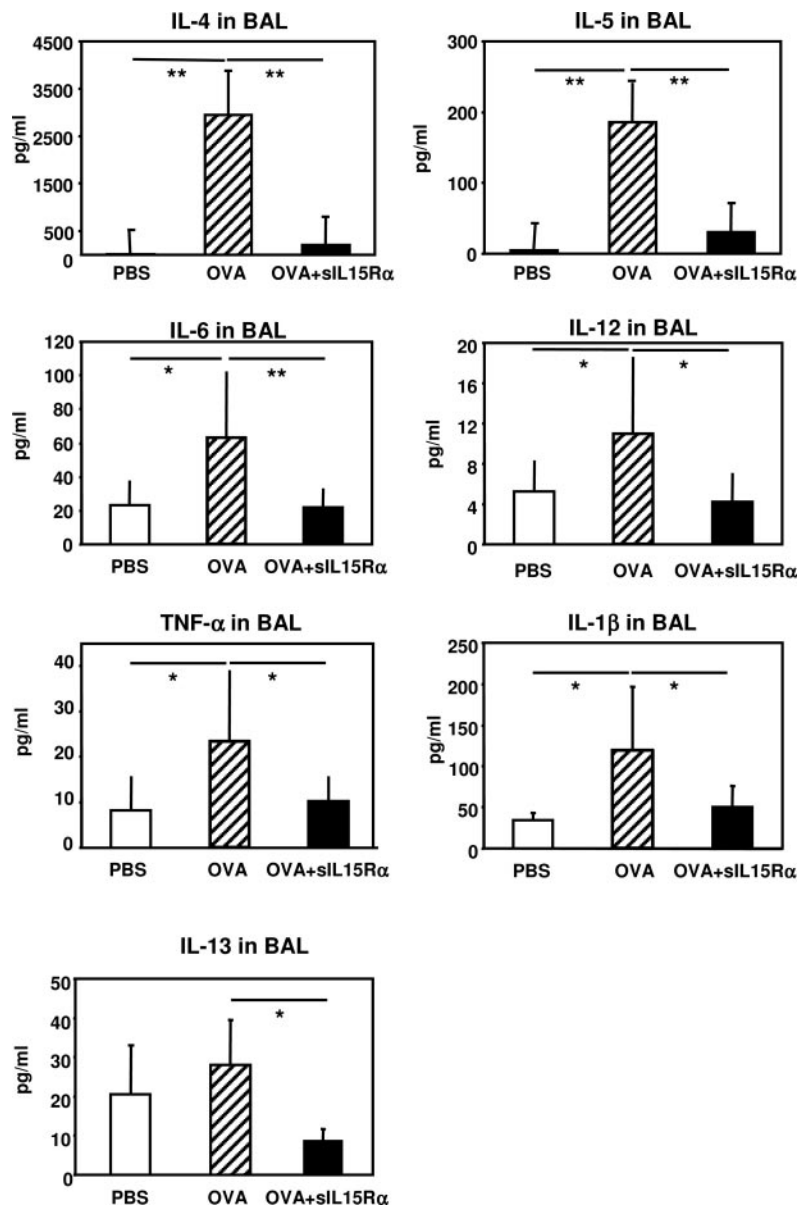


FIGURE 4. Treatment with high levels of sIL-15Rα significantly suppresses in vivo cytokine production. Cytokine concentrations were measured in cell-free BAL fluid. IL-4 was suppressed to control levels by sIL-15Rα ($p \leq 0.002$ compared with OVA treatment alone). In addition, IL-5 and IL-6 were significantly suppressed by sIL-15Rα application ($p \leq 0.01$ compared with OVA). Also the proinflammatory cytokines IL-12, TNF-α, IL-1β, and IL-13 were significantly reduced ($p \leq 0.05$).

complete absence of an allergic inflammation in OVA-sensitized, sIL-15Rα-treated mice (Fig. 2B). Eosinophils and lymphocytes were almost absent, comparable to unsensitized mice (highly significant reduction, $p \leq 0.003$; $p \leq 0.009$ compared with OVA alone). Neutrophils were slightly elevated in OVA- plus sIL-15Rα-treated mice compared with controls; however, they were also significantly suppressed compared with OVA application alone ($p \leq 0.02$), whereas alveolar macrophages again were represented at control levels ($p \leq 0.04$).

Histopathologic examination of lungs from OVA-sensitized mice revealed a pleiomorphic peribronchial and perivascular infiltrate consisting of eosinophils, lymphocytes, and neutrophils after OVA challenge that was not seen in control mice (Fig. 3). Treatment with sIL-15Rα prevented mice from the development of such pulmonary infiltration. In fact, no sign of inflammatory cell accumulation around the airways could be detected.

To address whether the failed induction of allergic inflammation is due to generalized systemic apoptosis induced by blocking IL-15, we performed additional experiments using the sensitization protocol described above and in addition one group of mice that were injected with the sIL-15Rα only without OVA. On day 17,

the number of apoptotic cells in the spleen and draining lymph nodes of the lung were analyzed by annexin V/propidium iodide staining and FACS analysis. However, the amount of apoptotic

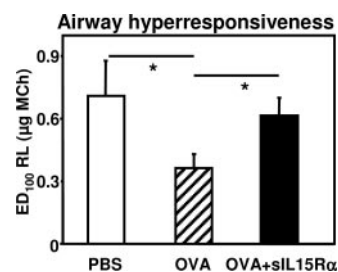


FIGURE 5. Soluble IL-15Rα reduces airway hyper-responsiveness. Twenty-four hours after the last OVA challenge, AHR was analyzed in response to MCh using intubated animals; the inhalational MCh dose (ED₁₀₀; micrograms) required to produce a 100% increase in lung resistance was markedly decreased in the OVA group compared with the control group. Treatment with sIL-15Rα reduced AHR significantly by 73%. Values are the mean $\pm$ SEM. *, $p < 0.05$ compared with OVA group ($n = 10$).

cells did not differ significantly in totally untreated mice, PBS controls, and the OVA-, OVA- plus sIL-15R α -, and sIL-15R α -injected mice (not shown). Because cell numbers in BAL (see Fig. 2) are at baseline levels in OVA- plus sIL-15R α -treated mice, it was not possible to detect apoptosis of eosinophils or other cells in the BAL directly. Hence, these data suggest that the reduced T cell inflammation and cell differentiation in sIL-15R α -treated mice (see below) are not due to general increased cell death. Taken together, these data strongly suggest that endogenous IL-15 plays an important role in the induction of allergic inflammation and attraction of inflammatory cells into the airways.

Soluble IL-15R α treatment prevents inflammatory cytokine release into the airways

During allergic inflammation, recruited inflammatory as well as airway epithelial cells and resident airway macrophages are activated and release cytokines, which, in turn, maintain this process by autocrine or paracrine mechanisms. Because T cells are almost absent after sIL-15R α treatment, the (mainly T cell-derived) Th2 cytokines IL-4 and IL-5 are only induced after OVA challenge in sensitized mice. In the presence of sIL-15R α , IL-4 and IL-5 levels in BAL were, as expected, comparable to control levels (Fig. 4; $p \leq 0.002$ and $p \leq 0.01$ compared with OVA treatment alone). IL-13 was found in low amounts, but, again, was significantly suppressed by sIL-15R α . In contrast, no significant differences in the (very low; <15 pg/ml) IFN- γ concentration in BAL were observed. Epithelial cells could be activated by a local inflammation (even in the absence of an inflammatory infiltrate). To determine whether IL-15 blockade could influence their proinflammatory cytokine release, we investigated concentrations of IL-6, IL-12, TNF- α , and IL-1 β in BAL fluid. Indeed, the production of these cytokines was significantly reduced (Fig. 4). Treatment of a separate group of mice with sIL-15R α alone did not induce any change in cytokine production above PBS control levels (not shown). Thus, blocking IL-15 in vivo not only inhibits cell recruitment to the site of inflammation, but also influences the cytokine milieu in the targeted tissue.

Soluble IL-15R α prevents the development of airway hyper-responsiveness

To detect the functional relevance of the reduced airway inflammation, lung function measurements were performed. Airway hyper-responsiveness in response to methacholine was assessed using intubated animals. The methacholine dose inducing an increase in resistance of 100% (ED₁₀₀) was used as readout parameter. The ED₁₀₀ was significantly decreased in OVA-sensitized animals compared with control animals, showing marked hyper-responsiveness ($p < 0.05$): 0.36 ± 0.07 vs 0.71 ± 0.17 μ g of MCh (mean $\pm$ SEM; see Fig. 5). Hyper-responsiveness was nearly totally prevented by sIL-15R α application: 0.62 ± 0.09 μ g of MCh ($p \leq 0.05$ compared with OVA). Therefore, sIL-15R α treatment prevents airway inflammation and decreases airway hyper-responsiveness.

Soluble IL-15R α treatment significantly suppresses OVA-specific Ab production

Sensitization to OVA induces specific Ab production, which depends on CD4⁺ T cell help and correlates with the degree of B cell activation. We previously demonstrated that supplementing IL-15 in vivo could accelerate and enhance Ab production up to 50-fold above that in solely OVA-sensitized mice (12). Therefore, we were interested in whether blocking IL-15 could suppress OVA-specific Ab production. Serum was collected after sIL-15R α treatment on day 10 and after OVA challenge on day 16, and serum OVA-specific Ab titers were measured. As shown in Fig. 6, production of OVA-specific IgE was inhibited significantly on day 10 ($p \leq$

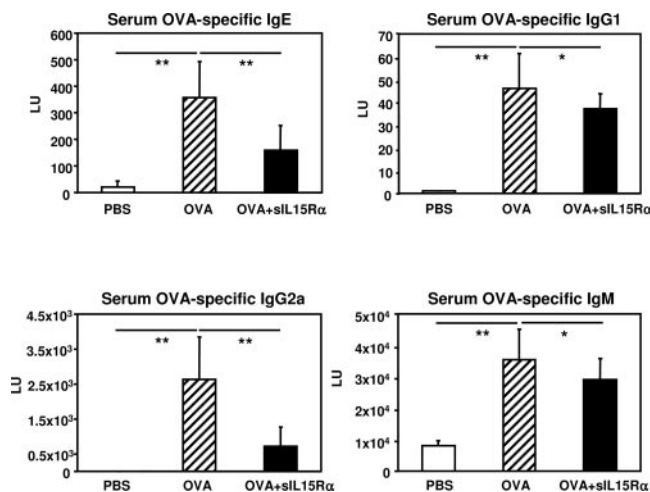


FIGURE 6. Soluble IL-15R α reduces anti-OVA Abs in vivo. OVA-specific serum Abs were measured using ELISA. Serum was collected after sIL-15R α treatment on day 10. Values are given in labor units (LU) as the mean $\pm$ SEM ($n = 9$ –17/group), and significance was calculated using Student's t test. *, $p \leq 0.05$; **, $p \leq 0.01$.

0.01). Specific IgG1 was suppressed significantly ($p \leq 0.05$) as were IgG2a ($p \leq 0.01$) and IgM ($p \leq 0.05$). Six days after sIL15R α treatment, significantly decreased IgE and IgG2b titers were still observed (not shown). In additional experiments, sIL-15R α was injected only three times, 30 min before sensitization to OVA on days 1, 7, and 14, which was sufficient to inhibit the Ab titers on day 17 (data not shown). Taken together, these data suggest that endogenous IL-15 is involved in the induction of allergic sensitization by increasing specific Ab production in this process.

Soluble IL-15R α treatment blocks CD8⁺ memory cell induction in vivo

Ag-specific T cells differentiate into memory cells, which persist for a long time in spleen and DLN and are characterized by high

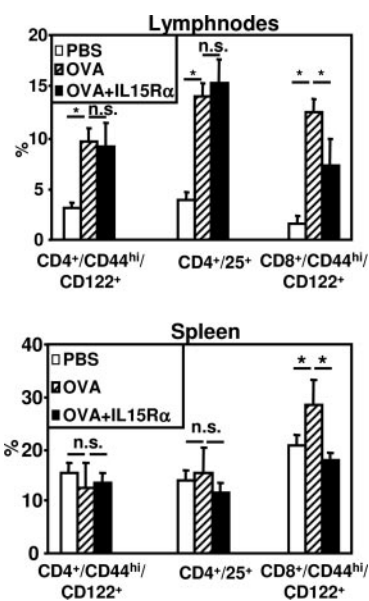


FIGURE 7. Soluble IL-15R α suppresses CD8⁺ memory cell generation in vivo. Lung DLN and spleen were analyzed by FACS for CD4⁺ and CD8⁺, CD44^{hi}/CD122⁺ memory cells as well as CD4⁺/CD25⁺ regulatory T cells on day 17. Significance was calculated using Student's t test. *, $p \leq 0.05$. One representative experiment is shown.

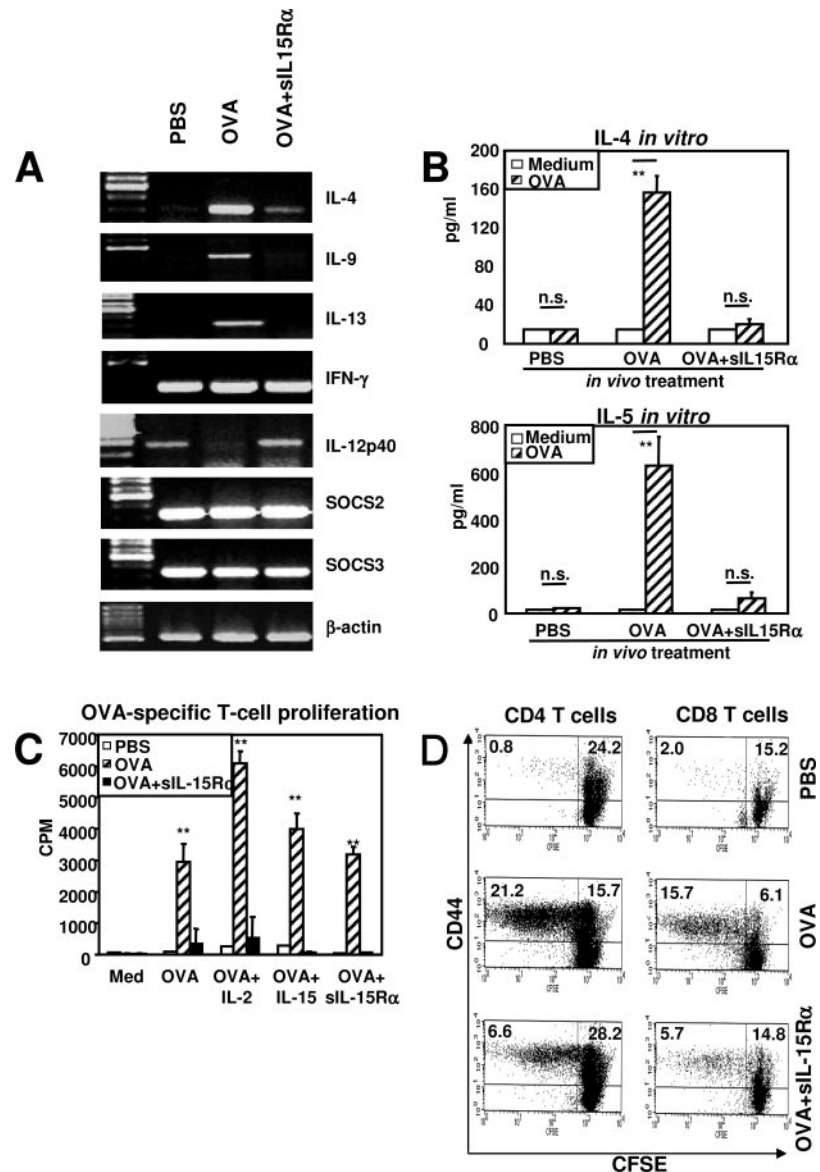


FIGURE 8. Soluble IL-15Rα blocks Th2 cytokine production and reduces Ag-specific proliferation in vitro. *A*, DLN were restimulated for 24 h with OVA, and RT-PCR for the indicated cytokine and suppressor of cytokine signaling (SOCS) mRNA expression was performed. *B*, Ex vivo OVA restimulation for 5 days of spleen cells from control mice, OVA-sensitized, or OVA- plus sIL-15Rα-treated mice. IL-4 and IL-5 were analyzed in cell culture supernatant. *C*, Isolated spleen cells were restimulated with OVA alone, OVA plus IL-2, OVA plus IL-15, or OVA plus sIL-15Rα at the indicated concentrations for 48 h, and proliferation was assessed by [^{3} H]thymidine incorporation for an additional 20 h. *D*, Isolated cells were labeled with CFSE and stimulated for 48 h with OVA. FACS analysis was performed on either CD4- or CD8- and CD44-stained, living cells. Given is the percentage of dividing cells.

expression of CD44 and CD122. Because it has been reported that IL-15 is essential for CD8⁺ memory differentiation, we investigated whether sIL-15Rα treatment modulates the development of these cells in vivo. FACS analysis on day 17 revealed that although CD8⁺ memory cells were indeed significantly reduced after sIL-15Rα treatment, no effect could be seen on CD4/CD122⁺ cell population (Fig. 7). Because CD4/CD25⁺ regulatory T cells are implicated in down-regulation of immune responses, we have analyzed these cells in OVA- and OVA- plus sIL-15Rα-treated groups. Despite their induction in DLN, no differences were observed. Total numbers of CD4 or CD8 T cells and B220 B cells in spleen and DLN were not affected by sIL-15Rα treatment (not shown), confirming previous findings after sIL-15Rα treatment in a Th1 DTH model (6). In addition, we performed experiments using the sensitization protocol described and one group of mice, which was injected with sIL-15Rα only without OVA. On day 17, the numbers of apoptotic cells in spleen and DLN of the lung were analyzed by annexin V/propidium iodide staining and FACS analysis. However, the amount of apoptotic cells did not differ significantly in totally untreated mice, PBS controls, and the OVA-, OVA- plus sIL-15Rα-, and sIL-15Rα-injected mice (not shown). This suggests that the reduced T cell differentiation in sIL-15Rα-

treated mice is not due to general increased cell death. Thus, these data indicate that blocking IL-15 in vivo inhibits the differentiation of T cells and suggest a close correlation between the suppressed allergic inflammation and the reduced number of CD8⁺ memory T cells.

Soluble IL-15Rα blocks the induction of Ag-specific Th2 T cells

To investigate the underlying mechanisms of how blocking IL-15 prevents induction of an allergic inflammation, we performed in vitro experiments using lymph node cells from treated mice. To determine whether induction of Ag-specific Th2 cells in vivo was blocked by sIL-15Rα, we analyzed the expression of Th2 cytokines in DLN after 24-h ex vivo OVA restimulation by RT-PCR. mRNA expression of the Th2 cytokines IL-4, IL-9, and IL-13 was very low or absent in sIL-15Rα-treated mice, whereas IFN-γ expression was comparable in all three groups. mRNA expression of the Th1 cytokine IL-12p40 was lower only in OVA-sensitized mice (Fig. 8A). In non-OVA-stimulated DLN cells, no Th2 message expression could be detected (not shown). Interestingly, sIL-15Rα treatment of the mice only twice on days 15 and 16 reduced IL-4 and IL-13 message expression comparable to the effect of 10-day treatment (not shown). The reduced Th2 phenotype after

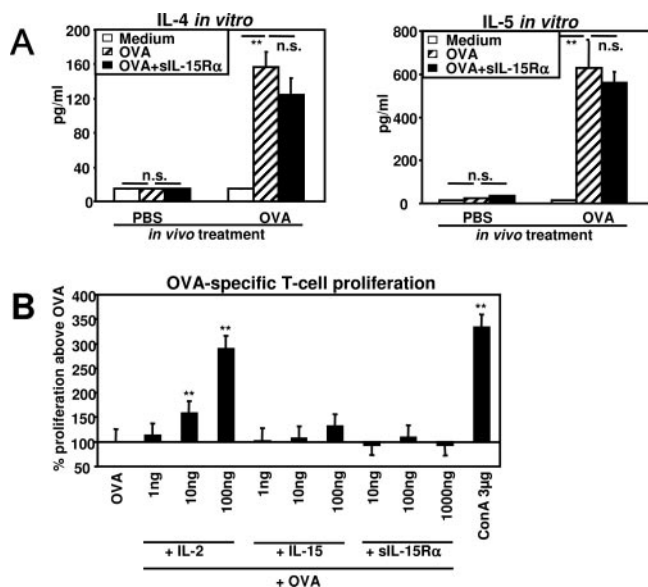


FIGURE 9. IL-15 is not involved in Ag-specific proliferation and cytokine production of already Th2-differentiated T cells in vitro. **A**, Ex vivo cytokine production of spleen cells from unsensitized or OVA-sensitized (not sIL-15R α -treated) mice isolated on day 17, after 5 days of stimulation with medium, OVA, or OVA plus sIL-15R α . **B**, Isolated spleen cells from OVA-sensitized (not sIL-15R α -treated) mice were incubated with OVA alone, OVA plus IL-2, OVA plus IL-15, or OVA plus sIL-15R α at the indicated concentrations for 48 h, and proliferation was assessed for an additional 20 h. Con A was used as a positive control for maximal proliferation. Given is the percent change (increase/decrease) compared with proliferation induced by OVA alone (which was set at 100%).

sIL-15R α treatment was not mediated by up-regulation of immunosuppressive suppressor of cytokine signaling-2 or -3 in DLN (Fig. 8A). We also stimulated T cells of these mice in vitro for 5 days with OVA and analyzed IL-4 and IL-5 secretion into the supernatant. As expected, cells from OVA-sensitized mice produced high amounts of these Th2 cytokines after restimulation with OVA (Fig. 8B). In contrast, cells from mice that had been treated in vivo with sIL-15R α failed to produce significant amounts of IL-4 and IL-5, corresponding to the suppressed message expression, as shown above. The failure in Th2 cytokine release was not mediated by enhanced Th1 differentiation, because no differences in IFN- γ release were observed (not shown). To further investigate this blocked induction of Ag-specific T cells, we analyzed these cells in a proliferation assay. Stimulation of OVA-sensitized cells induced a profound proliferation, which could be significantly increased by IL-2, but not by addition of IL-15 or blocked using sIL-15R α (Fig. 8C). Stimulating the cells from in vivo sIL-15R α -treated mice with OVA failed to induce significant [^{3} H]thymidine incorporation. Using Con A as a mitogen revealed strong T cell proliferation in all three groups, indicating that the effect is Ag specific and that sIL-15R α does not induce a general immunosuppression (not shown). To determine whether CD4 or CD8 T cells are specifically affected, the cells were labeled with CFSE and analyzed after 48-h stimulation with OVA. As shown in Fig. 8D, both CD4 and CD8/CD44 $^{+}$ cell proliferations were reduced by sIL-15R α treatment (Fig. 8D). Thus, in sIL-15R α -treated mice, the absence of allergic inflammation is accompanied by the absence of allergen-specific, Th2 cytokine-producing T cells.

IL-15 does not affect Ag-specific recall response in established, Th2-primed cells

From the data shown above, it was clear that blocking IL-15 interferes with the induction of Ag-specific Th2 cells in vivo. Therefore, to understand whether IL-15 is also able to modulate the recall response of already Th2-differentiated, allergen-specific T cells after repeated allergen contact, DLN cells from OVA-sensitized mice were stimulated in vitro with OVA alone or in the presence of IL-2, IL-15, or sIL-15R α . Cytokine production and proliferation were analyzed. As shown in Fig. 9A, OVA induced the expected IL-4 as well as IL-5 production. Adding sIL-15R α to OVA, however, did not significantly down-modulate Th2 cytokine release.

Finally, the Ag-specific proliferation of OVA-specific, already Th2-differentiated cells ex vivo and the modulatory effects of IL-15 (compared with IL-2) in this process were analyzed. OVA-induced proliferation was analyzed in cells from OVA-sensitized mice. Adding IL-2, but not IL-15, significantly increased proliferation compared with OVA alone (Fig. 9B). Interestingly, supplementing sIL-15R α to OVA, and thus blocking IL-15 again had no significant effect on OVA-induced proliferation (Fig. 9B). Con A was used as a positive control to induce maximal ex vivo T cell proliferation. These data support the assumption that IL-15 is essential for the induction phase of Th2 cell differentiation and memory T cells by APCs, but does not modulate (at least in vitro) Ag-specific (recall) T cell activation after repetitive contact of Ag-specific Th2 cells with the cognate Ag.

Discussion

We report in this study that systemic application of the IL-15 antagonist sIL-15R α blocks the differentiation of Th2-primed T cells. This inhibition in a model of allergic sensitization completely prevents the induction of local pulmonary allergic inflammation, including recruitment of inflammatory cells and development of bronchial hyper-responsiveness in vivo.

IL-15 is a T cell growth factor produced by a wide range of non-T cells, including epithelial cells (11), macrophages, and DCs (15). The capacity of mature DCs to prime naive T cells and to promote their differentiation is shaped by the secretion of T cell-activating cytokines, notably IL-15. In contrast, IL-15 also influences DC differentiation and maturation, as is evident, for example, by the IL-15-induced secretion of proinflammatory cytokines (15, 23) and increased expression of costimulatory molecules on APCs. It is well established that IL-15 is critically involved in the induction of T cell differentiation and, in particular, in the maintenance of CD8 $^{+}$ memory T cells in vivo, which is evident from their reduced number in IL-15 $^{-/-}$ and IL-15R α $^{-/-}$ mice (4, 5). Indeed, we recently showed that using IL-15 $^{-/-}$ mice or blocking IL-15 using the sIL-15R α also completely abrogated Th1 delayed-type hypersensitivity in two different models (6). In these models, the induction of Ag-specific CD8 $^{+}$ T cells critically depends on the expression of IL-15 by DC and the stimulation of these cells via an autocrine IL-15/IL-15R α loop. A similar mechanism may account for the block of Th2 differentiation we report in this study, when, in parallel to allergic sensitization, an IL-15 antagonist is injected in vivo. DC-derived cytokines are important for driving naive T cell differentiation and proliferation by IL-15. They also release proinflammatory cytokines such as TNF- α and IL-1, both under control of IL-15 (6), which up-regulates IL-2/IL15R β and the common γ -chain (24). Blocking IL-15, therefore, could inhibit DC activation (15) as well as the expansion of naive T cells, which might explain the observed lack of Th2 differentiation, as shown in this study.

In vitro restimulation of spleen cells from OVA-sensitized mice resulted in high Th2 cytokine production, which was absent in mice that had been treated in vivo with sIL-15R α . This suggests that no allergen-specific Th2 cells could be generated in the absence of IL-15 (which was not compensated by increased Th1 differentiation, because no enhanced IFN- γ release was detected). In contrast, if the Th2-differentiated, Ag-specific cells from OVA-sensitized mice were stimulated in vitro with OVA and sIL-15R α , no significant decrease in IL-4 and IL-5 production was evident compared with that after OVA alone, suggesting that IL-15 plays no role in the modulation of Ag recall stimulation of established Th2 cells with regard to cytokine production. This fact was also supported by findings reported by Dooms et al. (25), which elegantly demonstrated that IL-15 is more a survival factor for (established) memory CD4⁺ T cells than a promotor of CD4⁺ T cell expansion or DNA synthesis. IL-2, in contrast, induced a rapid and strong expansion when given during Ag stimulation.

IL-2 as well as IL-15 has been implicated in the generation or maintenance of so-called regulatory T cells. We found no differences in suppressive CD4/CD25⁺ T cells; however, it is currently not clear whether other regulatory mechanisms (immunosuppressive cytokines, such as IL-10 or TGF- β) or CD8 regulatory T cells are involved. It also remains to be clarified whether naive T cells during DC contact and Ag presentation in the absence of IL-15 fail to differentiate into effector/memory cells or become anergic, tolerogenic, or even are selectively eliminated by apoptosis.

Beside the effects on T cell activation and differentiation, IL-15 is a proinflammatory cytokine, with multiple targets during immune responses; therefore, blocking IL-15 in vivo might significantly reduce activation of the cells involved in allergic inflammation. The anti-inflammatory properties of the sIL-15R α we applied in this study have previously been shown in a model of collagen-induced arthritis (19), in which inflammation as well as Ab production were significantly reduced. Based on these findings, it was interesting to observe that inhibition of endogenous IL-15 could also suppress a Th2-type allergic inflammation. This reduction might also be mediated in part by blocking the chemotactic activity of (locally produced) IL-15 (26, 27), thus prohibiting recruitment of leukocytes at inflammation sites.

Besides DCs, IL-15 is mainly released by activated macrophages, which are abundant in the lung and activated during allergic and other inflammatory reactions. Alveolar macrophages from patients with inflammatory lung disease release elevated levels of IL-15 (28), suggesting a role as a proinflammatory cytokine in the lung, which could locally activate T cells or eosinophilic granulocytes, both of which are major contributors to allergic inflammation. Indeed, it has been demonstrated by immunohistology that IL-15-producing macrophages are more abundant in the submucosa of patients with severe asthma bronchiale and that IL-15 can act as a survival factor for human eosinophils in vitro (29) (our unpublished observations) and that the hypereosinophilic syndrome is associated with increased serum IL-15 levels (30). These findings point to the important role of IL-15 in eosinophil biology, which is supported by our data demonstrating that sIL-15R α significantly suppresses the influx of eosinophils as well as neutrophils and lymphocytes into the airway.

Interestingly, IL-15 plays an important role in Th2 reactions, in addition to the established Th2 cytokine IL-4. However, because sIL-15R α completely blocked Th1 reactions in vivo (6), IL-15 seems to be essential for the induction of T cell priming and differentiation in general, rather than being restricted to a particular Th1 or Th2 phenotype. The data presented in this study (and previous findings by others) argue against a specific role for IL-15 as

a Th1 or Th2 cytokine, but support its essential role in mediating inflammatory immune responses.

Another aspect of the block of allergic inflammation by sIL-15R α might be that the induction of an allergic inflammation in the lung by cross-linking IgE molecules on mast cells is reduced, because treatment with sIL-15R α also reduced the production of allergen-specific Ab. However, the suppression of Ab production again seemed not to be subtype specific; hence, all analyzed classes are significantly suppressed, indicating that IL-15 generally stimulates B cells, rather than induces any specific isotype class switch. That IL-15 is indeed an important mediator of B cell activation and Ab production has been shown by others (31, 32) and by our laboratory in previous studies, in which injection of IL-15 along with OVA could remarkably accelerate and enhance Ab production up to 50-fold (12). Therefore, allergen rechallenge could fail to induce a signal strong enough to induce complete mast cell activation (where IL-15 mediates IL-4 release and antiapoptotic signals (33, 34) and the initiation of proinflammatory cytokine and histamine release, leading to pulmonary inflammation.

In apparent contrast with the data presented in this study, a previous report (35) demonstrated that overexpression of IL-15 in transgenic mice in vivo suppressed the Th2-mediated allergic airway response. However, using IL-15 transgenic mice seems to be problematic for extrapolation of the results to normal immunopathology, because IL-15 is secreted in very high amounts in these transgenic mice. Normal wild-type mice, in contrast, do not secrete any detectable IL-15, as also demonstrated by Ishimitsu et al. (35) and many other groups. Subsequent to publication of this study, it became evident that membrane-bound IL-15 is important for mediating the effects of IL-15 in vivo (36), and not soluble IL-15, which until now has been difficult to detect in normal body fluids. For example, we have shown that specific priming of T cells depends on membrane expression of IL-15 and its interaction with IL-15R α on DC and not on secreted or released IL-15 (6).

Beside the injection of high dose sIL-15R α in a therapeutic attempt, we recently showed that several strains of mice constitutively secrete a soluble IL-15R α -chain (37). Many soluble cytokine receptors participate in the control of cytokine activity in vivo by inhibiting the local ability of cytokines to bind to their membrane receptors and generating a biological response, thus acting as natural antagonists (38). However, despite this well-documented endogenous antagonist activity, a clear definition of the roles and complex activities of endogenous soluble cytokine receptors in vivo is very difficult. The antagonistic effects of soluble cytokine receptors are directly related to their concentration and inversely related to the concentration of cytokines (i.e., the more cytokine present the more soluble receptor is required to prevent its binding to membrane receptors). Furthermore, the antagonistic properties are dependent on the binding affinities of the particular soluble cytokine receptors vs the functional high affinity membrane-bound receptor or receptor complex. For example, Sato et al. (39) demonstrated that exogenously administered sIL-4R can either potentiate or inhibit the activity of IL-4 in vivo depending on the relative molar ratios of sIL-4R to IL-4; low ratios promoted the cytokine activity, whereas an excess of sIL-4R over IL-4 inhibited responses to IL-4. Moreover, low levels of soluble TNF receptor appear to enhance TNF signaling, perhaps by stabilizing the ligand, whereas higher concentrations inhibited TNF activity (40). In agreement with these findings, exogenous sIL-15R α injected in high concentrations (50 μ g/mouse i.p.) inhibited the ability of IL-15 to boost allergic sensitization in vivo, acting as a natural antagonist, whereas circulating levels of endogenous sIL-15R α apparently were not sufficient for this task.

Taken together the results show that sIL-15R α is a potent, non-toxic inhibitor of allergic sensitization and pulmonary inflammation in a murine model of asthma bronchiale. Numerous studies have shown that the key pathological characteristics of allergic lung inflammation, including AHR and cellular inflammation, are not dependent upon one another. The advantage of blocking IL-15 is the broad action of this cytokine. Instead of one specific target or symptom in this study, we blocked a cascade of effector mechanisms, essential for allergic inflammation, starting at the level of Ag presentation by DCs, the differentiation of naive into effector/memory T cells, and the recruitment of leukocytes to inflammatory sites up to inhibition of activation and survival of mast cells and eosinophilic granulocytes.

Acknowledgments

We thank André Jenckel, Stefanie Belz, Maria-Virgilia Odenwald, and Jutta Schenck for excellent technical assistance, and Annette Wallisch for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

- Mosmann, T. R., and S. Sad. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 17:138.
- Haczku, A., R. Moqbel, M. Jacobson, A. B. Kay, P. J. Barnes, and K. F. Chung. 1995. T-cells subsets and activation in bronchial mucosa of sensitized Brown-Norway rats after single allergen exposure. *Immunology* 85:591.
- Hamelmann, E., A. Oshiba, J. Paluh, K. Bradley, J. Loader, T. A. Potter, G. L. Larsen, and E. W. Gelfand. 1996. Requirement for CD8⁺ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J. Exp. Med.* 183:1719.
- Kennedy, M. K., M. Glacum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191:771.
- Lodolce, J. P., D. L. Boone, S. Chai, R. E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 9:669.
- Rückert, R., K. Brandt, E. Bulanova, F. Mirghomizadeh, R. Paus, and S. Bulfone-Paus. 2003. Dendritic cell-derived IL-15 controls the induction of CD8 T cell immune responses. *Eur. J. Immunol.* 33:3493.
- Wilkinson, P. C., and F. Y. Liew. 1995. Chemoattraction of human blood T lymphocytes by interleukin-15. *J. Exp. Med.* 181:1255.
- Mori, A., M. Suko, O. Kaminuma, S. Inoue, T. Ohmura, Y. Nishizaki, T. Nagahori, Y. Asakura, A. Hoshino, Y. Okumura, et al. 1996. IL-15 promotes cytokine production of human T helper cells. *J. Immunol.* 156:2400.
- Bulfone-Paus, S., D. Ungureanu, T. Pohl, G. Lindner, R. Paus, R. Rückert, H. Krause, and U. Kunzendorf. 1997. Interleukin-15 protects from lethal apoptosis in vivo. *Nat. Med.* 3:1124.
- Lindner, G., R. Rückert, S. Bulfone-Paus, and R. Paus. 1998. Inhibition of chemotherapy-induced keratinocyte apoptosis in vivo by an interleukin-15-IgG fusion protein. *J. Invest. Dermatol.* 110:457.
- Rückert, R., K. Asadullah, M. Seifert, V. M. Budagian, R. Arnold, C. Trombott, R. Paus, and S. Bulfone-Paus. 2000. Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? *J. Immunol.* 165:2240.
- Rückert, R., U. Herz, R. Paus, D. Ungureanu, T. Pohl, H. Renz, and S. Bulfone-Paus. 1998. IL-15-IgG2b fusion protein accelerates and enhances a Th2 but not a Th1 immune response in vivo, while IL-2-IgG2b fusion protein inhibits both. *Eur. J. Immunol.* 28:3312.
- Morelli, A. E., A. F. Zahorchak, A. T. Larregina, B. L. Colvin, A. J. Logar, T. Takayama, L. D. Falo, and A. W. Thomson. 2001. Cytokine production by mouse myeloid dendritic cells in relation to differentiation and terminal maturation induced by lipopolysaccharide or CD40 ligation. *Blood* 98:1512.
- McInnes, I. B., J. al Mughales, M. Field, B. P. Leung, F. P. Huang, R. Dixon, R. D. Sturrock, P. C. Wilkinson, and F. Y. Liew. 1996. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat. Med.* 2:175.
- Mattei, F., G. Schiavoni, F. Belardelli, and D. F. Tough. 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. *J. Immunol.* 167:1179.
- Brandt, K., S. Bulfone-Paus, A. Jenckel, D. C. Foster, R. Paus, and R. Rückert. 2003. Interleukin-21 inhibits dendritic cell-mediated T cell activation and induction of contact hypersensitivity in vivo. *J. Invest. Dermatol.* 121:1379.
- Brandt, K., S. Bulfone-Paus, D. C. Foster, and R. Rückert. 2003. Interleukin-21 inhibits dendritic cell activation and maturation. *Blood* 102:4090.
- Niedbala, W., X. Wei, and F. Y. Liew. 2002. IL-15 induces type 1 and type 2 CD4⁺ and CD8⁺ T cells proliferation but is unable to drive cytokine production in the absence of TCR activation or IL-12 / IL-4 stimulation in vitro. *Eur. J. Immunol.* 32:341.
- Ruchatz, H., B. P. Leung, X. Q. Wei, I. B. McInnes, and F. Y. Liew. 1998. Soluble IL-15 receptor α -chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. *J. Immunol.* 160:5654.
- Herz, U., A. Braun, R. Rückert, and H. Renz. 1998. Various immunological phenotypes are associated with increased airway responsiveness. *Clin. Exp. Allergy* 28:625.
- Smith, X. G., E. M. Bolton, H. Ruchatz, X. Wei, F. Y. Liew, and J. A. Bradley. 2000. Selective blockade of IL-15 by soluble IL-15 receptor α -chain enhances cardiac allograft survival. *J. Immunol.* 165:3444.
- Glaab, T., W. Mitzner, A. Braun, H. Ernst, R. Korolewitz, J. M. Hohlfeld, N. Krug, and H. G. Hoymann. 2004. Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *J. Appl. Physiol.* 37:1104.
- Ohteki, T., K. Suzue, C. Maki, T. Ota, and S. Koyasu. 2001. Critical role of IL-15-IL-15R for antigen-presenting cell functions in the innate immune response. *Nat. Immunol.* 2:1138.
- Geginat, J., F. Sallusto, and A. Lanzavecchia. 2001. Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4⁺ T cells. *J. Exp. Med.* 194:1711.
- Dooms, H., M. Desmedt, S. Vancaeneghem, P. Rottiers, V. Goossens, W. Fiers, and J. Groten. 1998. Quiescence-inducing and antiapoptotic activities of IL-15 enhance secondary CD4⁺ T cell responsiveness to antigen. *J. Immunol.* 161:2141.
- Jonuleit, H., K. Wiedemann, G. Muller, J. Degwert, U. Hoppe, J. Knop, and A. H. Enk. 1997. Induction of IL-15 messenger RNA and protein in human blood-derived dendritic cells: a role for IL-15 in attraction of T cells. *J. Immunol.* 158:2610.
- Sancho, D., M. Yanez-Mo, R. Tejedor, and F. Sanchez-Madrid. 1999. Activation of peripheral blood T cells by interaction and migration through endothelium: role of lymphocyte function antigen-1/intercellular adhesion molecule-1 and interleukin-15. *Blood* 93:886.
- Zissel, G., I. Baumer, M. Schlaak, and J. Müller-Quernheim. 2000. In vitro release of interleukin-15 by broncho-alveolar lavage cells and peripheral blood mononuclear cells from patients with different lung diseases. *Eur. Cytokine Network* 11:105.
- Hoontrakoon, R., H. W. Chu, S. J. Gardai, S. E. Wenzel, P. McDonald, V. A. Fadok, P. M. Henson, and D. L. Bratton. 2002. Interleukin-15 inhibits spontaneous apoptosis in human eosinophils via autocrine production of granulocyte macrophage-colony stimulating factor and nuclear factor- κ B activation. *Am. J. Respir. Cell Mol. Biol.* 26:404.
- Means-Markwell, M., T. Burgess, D. deKeraty, K. O'Neil, J. Mascola, T. Fleisher, and D. Lucey. 2000. Eosinophilia with aberrant T cells and elevated serum levels of interleukin-2 and interleukin-15. *N. Engl. J. Med.* 342:1568.
- Armitage, R. J., B. M. Macduff, J. Eisenman, R. Paxton, and K. H. Grabstein. 1995. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J. Immunol.* 154:483.
- Bulanova, E., V. Budagian, T. Pohl, H. Krause, H. Dürkop, R. Paus, and S. Bulfone-Paus. 2001. The IL-15R α chain signals through association with Syk in human B cells. *J. Immunol.* 167:6292.
- Masuda, A., T. Matsuguchi, K. Yamaki, T. Hayakawa, M. Kubo, W. J. LaRoche, and Y. Yoshikai. 2000. Interleukin-15 induces rapid tyrosine phosphorylation of STAT6 and the expression of interleukin-4 in mouse mast cells. *J. Biol. Chem.* 275:29331.
- Masuda, A., T. Matsuguchi, K. Yamaki, T. Hayakawa, and Y. Yoshikai. 2001. Interleukin-15 prevents mouse mast cell apoptosis through STAT6-mediated Bcl-x_L expression. *J. Biol. Chem.* 276:26107.
- Ishimitsu, R., H. Nishimura, T. Yajima, T. Watase, H. Kawachi, and Y. Yoshikai. 2001. Overexpression of IL-15 in vivo enhances Tc1 response, which inhibits allergic inflammation in a murine model of asthma. *J. Immunol.* 166:1991.
- Dubois, S., J. Mariner, T. A. Waldmann, and Y. Tagaya. 2002. IL-15R α recycles and presents IL-15 in trans to neighboring cells. *Immunity* 17:537.
- Budagian, V., E. Bulanova, Z. Orinska, A. Ludwig, S. Rose-John, P. Saftig, E. C. Borden, and S. Bulfone-Paus. 2004. Natural soluble interleukin-15R α is generated by cleavage that involves the tumor necrosis factor- α -converting enzyme (TACE/ADAM17). *J. Biol. Chem.* 279:40368.
- Fernandez-Botran, R. 2000. Soluble cytokine receptors: novel immunotherapeutic agents. *Expert. Opin. Invest. Drugs* 9:497.
- Sato, T. A., M. B. Widmer, F. D. Finkelman, H. Madani, C. A. Jacobs, K. H. Grabstein, and C. R. Maliszewski. 1993. Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses in vivo. *J. Immunol.* 150:2717.
- Heaney, M. L., and D. W. Golde. 1996. Soluble cytokine receptors. *Blood* 87:847.