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Effect of interleukin-16-blocking peptide on parameters of allergic asthma in a murine model

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Abstract

In this study, we examined whether peptides based on the hydrophilic Cluster of Differentiation (CD) 4-binding part of the amino acid sequence of human interleukin-16 can block interleukin-16-induced chemotaxis of murine lymphocytes in vitro. Peptide 3 was capable of inhibiting interleukin-16-induced chemotaxis of murine splenocytes in vitro. Next, we compared the effects of intra-airway administration of peptide 3 with those of antibodies to interleukin-16 on antigen-induced features in a murine model of allergic asthma. Intra-airway administration of peptide 3 largely inhibited the development of antigen-induced airway hyperresponsiveness while airway eosinophilia was not affected. Similar effects were observed after intranasal application of antibodies to interleukin-16. These results indicate that treatment with peptide 3 causes the same effects as do antibodies to interleukin-16, possibly via the inhibition of interaction between interleukin-16 and its receptor CD4. Therefore, peptide 3 could be useful as a lead compound in attempting to limit airway hyperresponsiveness via binding to CD4.

Keywords: Eosinophil; Hyperresponsiveness; Asthma; Interleukin-16; (Murine)

1. Introduction

Human allergic asthma is characterised by airway hyperresponsiveness and infiltration of lymphocytes and eosinophils in the lungs (Corrigan and Kay, 1992). There is increasing evidence that $CD4^+$ T cells play a crucial role in orchestrating these different phenomena by producing Th2-type cytokines, including interleukin-4 and interleukin-5 (Corrigan and Kay, 1992; Virchow et al., 1996).

Besides interleukin-4 and interleukin-5, many other cytokines have been associated with the pathology of asthma, one of them being interleukin-16 (Center et al., 1996). Interleukin-16 has been demonstrated to use the CD4 molecule as its receptor. Upon binding and cross-linking of CD4 molecules, several second messengers, including p56lck and protein kinase C, are activated (Haughn et al., 1992; Ryan et al., 1995; Racioppi et al., 1996). Furthermore, interleukin-16 can evoke different functional responses in $CD4^+$ cells. One of the most extensively described actions of CD4 cross-linking by interleukin-16 is the induction of chemotaxis in vitro (Center and Cruikshank, 1982). It has been demonstrated that interleukin-16 can induce such responses in various $CD4^+$ cells, including eosinophils, monocytes and T helper cells (Center et al., 1995, 1996). Other biological effects of interleukin-16 include induction of interleukin-2R expression and up-regulation of MHC-II expression in human lymphocytes (Center et al., 1995, 1996).

Both $CD4^+$ and $CD8^+$ T cells constitutively express messenger RNA for interleukin-16 and the biologically active protein is secreted from $CD8^+$ T cells after stimulation with either histamine or 5-hydroxytryptamine (5-HT) (Laberge et al., 1995, 1996). Furthermore, $CD4^+$ T cells release interleukin-16 after mitogen, antigen or anti-CD3 stimulation (Center et al., 1996). Bioactive interleukin-16 can also be produced by eosinophils, mast cells and epithelial cells (Bellini et al., 1993; Lim et al., 1996; Rumsaeng

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et al., 1997). These data suggest that interleukin-16 may be involved in the pathophysiology of asthma. Indeed, in patients with allergic asthma as well as in a murine model of allergic asthma, there is interleukin-16 expression in epithelial cells and bioactive interleukin-16 was found in bronchoalveolar lavage fluid after antigen challenge (Laberge et al., 1997; Hessel et al., 1998). Interestingly, inhibition of endogenous interleukin-16 by intraperitoneal administration of antibodies to interleukin-16 has been demonstrated to partially decrease airway hyperresponsiveness but not to affect the number of eosinophils in bronchoalveolar lavage fluid in a murine model of allergic asthma (Hessel et al., 1998).

In a previous study, Keane et al. (1998) demonstrated that peptide 3, which is based on the predicted amino acid sequence of one of the hydrophilic C-terminal regions of interleukin-16, is capable of partially inhibiting recombinant human interleukin-16-induced chemotaxis of peripheral blood mononuclear cells. They also demonstrated that peptide 3 could displace binding of antibodies (OKT4) to CD4 molecules. These data suggest that the C-terminal hydrophilic domain of interleukin-16 is involved in binding to CD4 and is critical for induction of chemotaxis in $CD4^+$ cells (Hessel et al., 1998). Additionally, these data suggest that the peptide might inhibit interleukin-16-induced activity by binding to CD4 molecules, thereby preventing cross-linking of CD4 molecules by interleukin-16. Finally, sequence homology of murine and human interleukin-16 is over 80% and cross-specificity is nearly 100% since identical effects were observed on migration of human or murine $CD4^+$ T lymphocytes induced by either recombinant human or recombinant murine interleukin-16 and this chemoattracting activity could be blocked by anti-human interleukin-16 mAb (clone 14.1, Keane et al., 1998).

In the present study, we examined the effect of various peptides based on the predicted amino acid sequence of interleukin-16 on recombinant human interleukin-16-induced chemotaxis of lymphocytes in vitro. Next, we examined the effects of intranasal administration of these peptides on airway hyperresponsiveness and eosinophilia in a murine model of asthma. In addition, we compared the effects of local administration of these peptides with those of administration of antibodies to interleukin-16 on the same parameters.

2. Materials and methods

2.1. Peptides and antibodies

Synthetic oligopeptides corresponding to three hydrophilic domains identified within the human interleukin-16 sequence were used in this study. The single letter amino acid codes of the different peptides are: MPDLNSSTDSA (based on amino acids 502–512, Fig. 1), designated peptide 1, AASEQSETVQPGDEIL (based on amino acids 569–584, Fig. 1), designated peptide 2 and RRKSLQSKETTAAGDS, peptide 3 (based on amino acids 616–631, Fig. 1). Peptides were generously provided by Dr. W.W. Cruikshank and produced as previously described (Keane et al., 1998). Monoclonal antibodies to human interleukin-16 (clone 14.1) were verified by western blotting with recombinant human interleukin-16 and shown to be neutralising by inhibition of chemoattractant activity, interleukin-2 receptor expression and human immunodeficiency virus (HIV)-1 repression in interleukin-16-treated cells (Cruikshank et al., 1994; Theodore et al., 1996). Finally, sequence homology of murine and human interleukin-16 is over 80% and cross-specificity is nearly 100% since identical effects were observed on migration of human or murine $CD4^+$ T lymphocytes by either recombinant human or recombinant murine interleukin-16 and this chemoattracting activity could be blocked by anti-human interleukin-16 mAb (clone 14.1, Keane et al., 1998).

2.2. Cell isolation and chemotaxis assay

Murine splenocytes were isolated from killed BALB/c mice and maintained in RPMI 1640 medium (Bio-Whittaker, Walkersville, MD) supplemented with 10% fetal calf serum and 100 units ml^{-1} of both penicillin and streptomycin (complete medium). Erythrocytes were lysed by suspension in one part complete medium to three parts Gey's solution. This mixture was incubated on ice for 2 min. The reaction was stopped by the addition of 10 parts complete medium and the cells were then washed twice in complete medium before use in experiments. Chemotaxis was performed using a modified Boyden chamber assay as described previously (Center and Cruikshank, 1982; Cruikshank and Center, 1982). Cells were suspended at 5×10^6 ml^{-1} in complete medium and incubated for 15 min with or without the different peptides at a concentration of 5 μg ml^{-1}. A nitrocellulose membrane with a pore size of 12 μm separated cells in the upper wells from control buffer or experimental supernatants in the lower wells. Supernatants in the lower wells consisted of recombinant murine interleukin-16 (100 nM) or medium alone. Chambers were incubated at 37°C for 4 h, then the membranes were removed, stained with hematoxylin and dehydrated by sequential washes in ethanol, propanol, and finally xylene. Cell migration was quantitated by light microscopy, with counts of the number of cells migrating below a depth of 50 μm. All samples were tested in duplicate, and four high-power fields were examined in each duplicate. Chemotaxis induced by medium alone was taken as 100%.

2.3. Sensitisation and challenge

Specified pathogen-free male BALB/c mice (age 6–8 weeks) were obtained from the breeding colony of the

1 MDYDFDTTAE DPWVRISDCI KNLFSPIMSE NHGHMPLQPN ASLNEEEGTQ GHPDGTPPKL

61 DTANGTPKVY KSADSSTVKK GPPVAPKPAW FRQSLKGLRN RASEPRGLDP PALSTQPAPA

121 SREHLGSHIR ASSSSSSIRQ RISSFETFGS SQLPDKGAQR LSLQPSSGEA AKPLGKHEEG

181 RFSGLLGRGA APTLVPQQPE QVLSSGSPAA SEARDPGVSE SPPPGRQPNQ KTFPPGPDPL

241 LRLLSTQAEE SQGPVLKMPS QRARSFPLTR SQSCETKLLD EKTSKLYSIS SQVSSAVMKS

301 LLCLPSSISC AQTPCIPKAG ASPTSSSNED SAANGSAETS ALDTGFSLNL SELREYTEGL

361 TEAKEDDDGD HSSLQSGQSV ISLLSSEELK KLIEEVKVLD EATLKQLDGI HVTILHKEEG

421 AGLGFSLAGG ADLENKVITV HRVFPNGLAS QEGTIQKGNE VLSINGKSLK GTTHHDALAI

481 LRQAREPRQA VIVTRKLTPE AMPDLNSSTD SAASASAASD VSVESTAEAT VCTVTLEKMS

peptide 1

541 AGLGFSLEGG KGSLHGDKPL TINRIFKGAA SEQSETVQPG DEILQLGGTA MQGLTRFEAW

peptide 2

601 NIIKALPDGP VTIVIRRKSL QSKETTAAGDS

peptide 3

Fig. 1. Alignment of the predicted human pro-IL16 amino acid sequence of the longest open reading frame (Adapted from Keane et al., 1998). Genbank database accession number M90391. Underlined sequences represent the predicted amino acid sequences on which the different oligopeptides were based.

Central Animal Laboratory (The Netherlands). All experiments were approved by the Animal Care Committee of Utrecht University. The mice were housed in Macrolon cages and provided with food and water ad libitum. Active sensitisation was obtained with seven intraperitoneal injections of 10 μg ovalbumin (grade V) in 0.5 ml pyrogen free saline on alternate days. Four weeks after the last injection, the mice were exposed to either eight ovalbumin (2 mg ml^{-1} in saline) or saline aerosols for 5 min on consecutive days (1 aerosol/day). The aerosols were generated with a jet nebulizer (Pari IS-2, Pari-Werk, Starnberg, Germany) connected to a Plexiglas exposure chamber with a volume of 5 l in which a maximum of six animals were placed. Approximately 30 min before each antigen exposure, the mice were treated intranasally with peptides based on the predicted sequence of interleukin-16 (Keane et al., 1998) after inhalation of a short-lasting anaesthetic (Halothane, ALBIC, Maassluis, The Netherlands). Peptides 1 and 3 were tested at a dose of 100 μg day^{-1}. In a different series of experiments, peptides 2 and 3 were tested at higher doses (240 μg day^{-1}).

The effects of local administration of anti-human interleukin-16 or control antibodies were also determined. In this experiment, animals were treated intranasally on the first and fifth day of the challenge period with either murine immunoglobulin (IgG) 2a or anti-interleukin-16 monoclonal antibodies (500 μg/mouse) 30 min before the antigen or saline challenge.

2.4. Airway responsiveness in vivo

Airway responsiveness was measured in vivo 24 h after the last aerosol exposure, using a modified plethysmograph

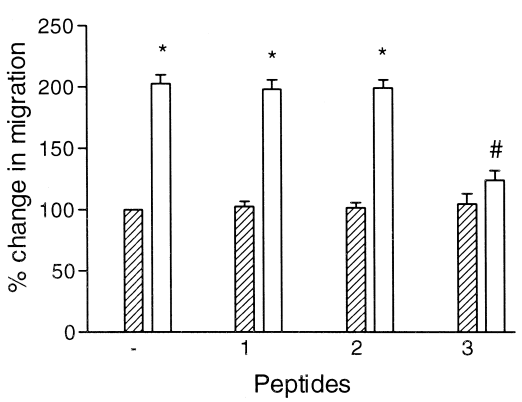

Fig. 2. The effects of different peptides on IL16-induced (100 nM) chemotaxis (open bars) was tested. Chemotaxis of mouse splenocytes was compared to that in complete medium (hatched bars, -) or to the effect of the different peptides without IL16 (hatched bars, 1, 2 or 3). Results are expressed as means ± S.E.M. $^{*}P < 0.05$ significantly different from control chemotaxis induced by medium or by the different peptides alone. # $P < 0.05$ significantly different from IL16-induced chemotaxis.

as described previously (De Bie et al., 1998). In short, mice were anaesthetised by intraperitoneal injection of urethane (2 g kg^{-1}), and placed on a heated blanket (30°C). The trachea and jugular vein were cannulated. Spontaneous breathing of the animals was suppressed by i.v. injection of tubocurarine chloride (3.3 mg kg^{-1}). The tracheal cannula was attached to a ventilator (model 687, Harvard Apparatus, Southnatick, MA, USA). The mice maintained physiological arterial blood gas parameters (data not shown). Changes in flow and pressure were measured using a plethysmograph coupled to pressure transducers (M45, Validyne Engineering Corp. Northridge, CA, USA). Lung resistance (R_L) was measured by quantitating $\Delta V/\Delta P_t$ (ΔP_t = change in tracheal pressure, ΔV = change in flow) at points of equal volume (70% tidal volume) using specific software (Model 6, Buxco, Sharon, CT, USA). After the response had returned to its baseline level (after at least 4 min), doses of methacholine ranging from 40 to 640 mg kg^{-1} were administered via the jugular catheter. For each dose of methacholine, the increase in airway resistance was measured at its peak and expressed in cm H_2O ml^{-1} s^{-1}. At least six mice were evaluated per experimental group.

2.5. Bronchoalveolar lavage

Bronchoalveolar lavage was performed in the same animals that were used for airway hyperresponsiveness measurements. Mice were lavaged five times through the tracheal cannula with 1-ml aliquots of pyrogen-free saline at 37°C. The bronchoalveolar lavage cells were washed with cold phosphate-buffered saline (PBS, 400 × *g*, 4°C, 5

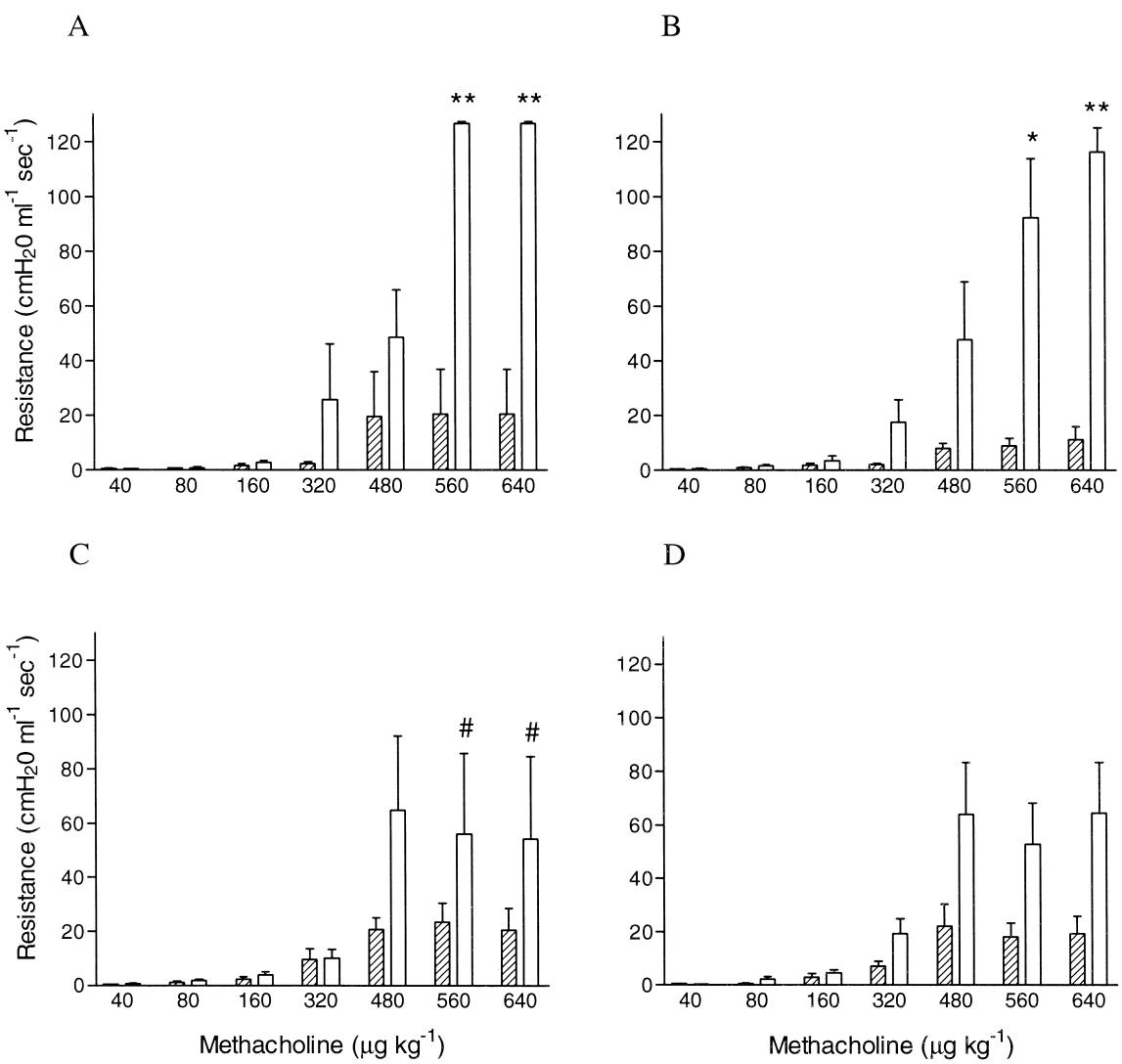

Fig. 3. Airway resistance after intravenous administration of methacholine in ovalbumin-sensitised mice at 24 h after the last challenge with saline (hatched bars) or ovalbumin (open bars) and intranasally treated daily during the challenge period with control peptides (100 μg day^{-1}, A, or 240 μg day^{-1}, B). Furthermore, animals were treated with peptide 3 (100 μg day^{-1}, C, or 240 μg day^{-1}, D). Results are expressed as means ± S.E.M. for five to six animals per group. $^{*}P < 0.05$; $^{**}P < 0.01$ significantly different from saline-challenged animals. # $P < 0.05$ significantly different from control peptide-treated ovalbumin-challenged animals.

min) and the pellet was resuspended in 200 μl cold PBS. The total number of bronchoalveolar lavage cells was counted with a Bürker–Türk chamber. For differential bronchoalveolar lavage cell counts, cytospin preparations were made and stained with Diff-Quick (Merz & Dade, Düdingen, Switzerland). After coding, all cytospin preparations were evaluated by one observer using oil immersion microscopy. Cells were identified and differentiated into mononuclear cells, neutrophils and eosinophils by standard morphology. At least 200 cells per cytospin preparation were counted and the absolute number of each cell type was calculated.

2.6. Materials

Ovalbumin (chicken egg albumin crude grade V) was purchased from Sigma (St. Louis, MO, USA). Urethane and methacholine (acetyl-ß-methylcholine) were purchased from Janssen Chimica (Beerse, Belgium), tubocurarine chloride from Nogepha (The Netherlands) and Tween-20 from Merck (Darmstadt, Germany).

2.7. Statistical analysis

Total bronchoalveolar lavage cell number and the numbers of the various bronchoalveolar lavage cell types were tested with an analysis of variance (ANOVA). For cell types with low numbers, e.g., eosinophils or neutrophils, a Poisson distribution was assumed. Data on airway responsiveness were tested for significance using ANOVA followed by post-hoc comparison using the Bonferroni test. The data are expressed as means ± S.E.M. and a difference was considered to be significant when $P < 0.05$. Statistical analyses were carried out using SPSS/PC$^+$ (SPSS, Chicago, IL).

3. Results

3.1. Chemotaxis assay

The effects of peptides 1, 2 and 3 were measured on murine recombinant interleukin-16-induced mouse lymphocyte motility. Lymphocyte motility induced by medium alone was taken as 100%. The interleukin-16-induced chemoattractive activity amounted to 203 ± 7%, which was significantly ($P < 0.05$) inhibited by peptide 3 to 124 ± 8% (Fig. 2). Incubation of the target cells with either peptide 1 or 2 did not result in any detectable decrease in interleukin-16-induced chemotaxis (Fig. 2). Furthermore, peptides 1, 2 and 3 did not induce any chemotaxis by themselves as compared to chemotaxis induced by medium alone (Fig. 2).

3.2. Airway resistance in vivo

Peptides 1 and 2 did not inhibit chemotaxis in vitro (Fig. 2) and were therefore used as control peptides in the in vivo studies. Peptide 1 was used as control peptide at a dosage of 100 μg day^{-1}, whereas peptide 2 was used as control at a higher dose (240 μg day^{-1}). In animals treated daily intranasally with control peptides, airway hyperresponsiveness after ovalbumin challenge was observed when compared with saline-challenged animals. After treatment with control peptide (100 μg day^{-1}), airway resistance in ovalbumin-challenged animals was significantly ($P < 0.01$) enhanced to 127 ± 1 cm H_2O ml^{-1} s^{-1}, as compared to that in saline-challenged ani-

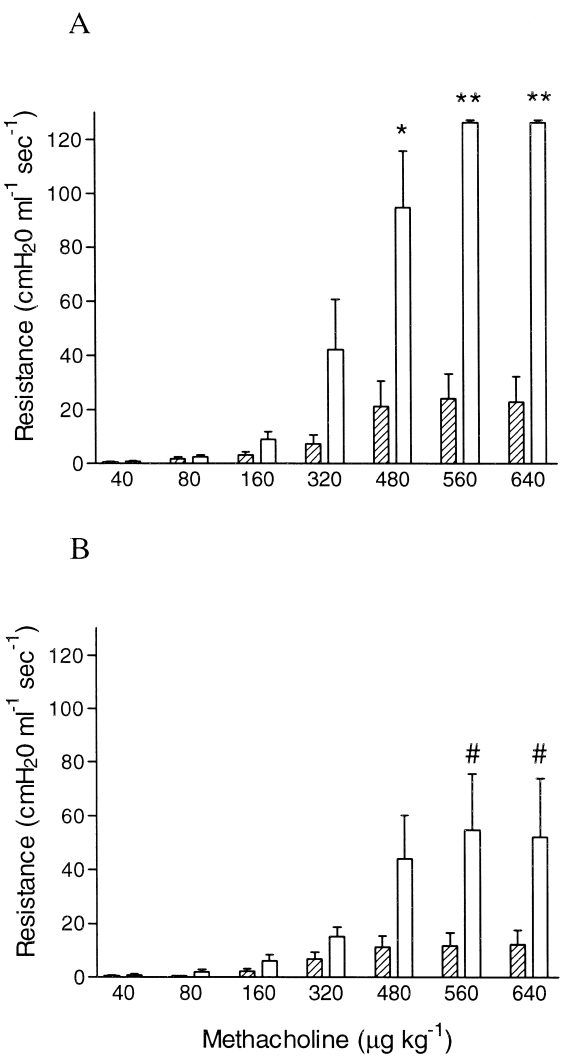

Fig. 4. Airway resistance after intravenous administration of methacholine in ovalbumin-sensitised mice at 24 h after the last challenge with saline (hatched bars) or ovalbumin (open bars) and treated intranasally on the first and fifth day of the challenge period with control antibody (mIgG2a, A) or antibodies to IL16 (500 μg/animal, B). Results are expressed as means ± S.E.M. for five to six animals per group. $^*P < 0.05$; $^{**}P < 0.01$ significantly different from saline-challenged animals. # $P < 0.05$ significantly different from mIgG2a-treated ovalbumin-challenged animals.

mals (20 ± 16 cm H_2O ml^{-1} s^{-1}, Fig. 3A). In animals treated with control peptide at a higher dose (240 μg day^{-1}), airway resistance in response to 640 μg kg^{-1} methacholine amounted to 116 ± 9 cm H_2O ml^{-1} s^{-1} after ovalbumin challenge, whereas in saline-challenged animals airway resistance amounted to 11 ± 5 cm H_2O ml^{-1} s^{-1} ($P < 0.05$, Fig. 3B). Treatment with peptide 3 (100 μg day^{-1}), however, significantly ($P < 0.05$) reduced the ovalbumin-induced airway hyperresponsiveness by 57% to 54 ± 30 cm H_2O ml^{-1} s^{-1} at the highest dose of methacholine, as compared to that in ovalbumin-challenged control peptide-treated mice (Fig. 3C). A higher dose of peptide 3 (240 μg day^{-1}) did not further reduce the airway resistance to methacholine (64 ± 19 cm H_2O ml^{-1} s^{-1} at the highest dose of methacholine, Fig. 3D). No significantly different effects were observed between all differently treated saline-challenged animals.

In animals treated intranasally with mIgG2a and challenged with saline, airway responses to the highest dose of methacholine (640 μg kg^{-1}) amounted to 23 ± 10 cm H_2O ml^{-1} s^{-1}, whereas in ovalbumin-challenged animals, airway responses were significantly ($P < 0.01$) enhanced to 126 ± 1 cm H_2O ml^{-1} s^{-1} (Fig. 4A). Treatment of ovalbumin-challenged mice with antibodies to interleukin-16 (500 μg at day 1 and 5) significantly decreased airway resistance by 59%, when compared to that in mIgG2a-treated ovalbumin-challenged animals. However, this airway resistance was still significantly ($P < 0.05$) enhanced as compared to that in saline-challenged anti-interleukin-16-treated animals (12 ± 5 cm H_2O ml^{-1} s^{-1}, Fig. 4B).

Table 1
Percentage of eosinophils present in bronchoalveolar lavage fluid from ovalbumin-sensitised and saline or ovalbumin-challenged mice. During the challenge period, the mice were treated intranasally daily with control peptide or peptide 3 (100 μg day^{-1}, 30 min before each aerosol challenge). In a different experiment, mice were treated daily during the challenge period with either control peptide or peptide 3 (240 μg day^{-1}, 30 min before each aerosol challenge) or with either mIgG2a or antibodies to IL16 (500 μg $mouse^{-1}$ on the first and fifth day of the challenge period).
Data are expressed as means ± S.E.M.

Treatment/challenge	Dosage (μg day^{-1})	Eosinophils (%)
Control/saline	100	2 ± 1
Control/ovalbumin	100	42 ± 11[a]
Peptide 3/saline	100	1 ± 1
Peptide 3/ovalbumin	100	54 ± 10[a]
Control/saline	240	1 ± 0
Control/ovalbumin	240	14 ± 2[a]
Peptide 3/saline	240	1 ± 0
Peptide 3/ovalbumin	240	23 ± 5[a]
mIgG2a/saline	500	0 ± 0
mIgG2a/ovalbumin	500	14 ± 9[a]
Anti-IL16/saline	500	2 ± 1
Anti-IL16/ovalbumin	500	21 ± 8[a]

[a] $P < 0.05$ significantly different from saline-challenged animals.

3.3. Cellular infiltration into bronchoalveolar lavage fluid

No or almost no eosinophils could be detected in bronchoalveolar lavage fluid from saline-challenged animals treated with the peptides, mIgG2a or anti-interleukin-16 (Table 1). In all differently treated animals, ovalbumin challenge caused infiltration of eosinophils into the bronchoalveolar lavage fluid (Table 1). No significant effects of the different treatments of ovalbumin-challenged animals on this eosinophilic infiltration were detected. Furthermore, no significant differences in infiltration of mononuclear cells or neutrophils were observed between different treatment groups (data not shown). Finally, no differences were observed in total number of cells between the different treatment groups (data not shown).

4. Discussion

The study now described showed that peptide 3, which is based on the predicted amino acid sequence of recombinant human interleukin-16 is capable of inhibiting recombinant murine interleukin-16-induced chemotaxis of murine splenocytes. Peptide 3 also markedly inhibited antigen-induced airway hyperresponsiveness in a murine model of allergic asthma, whereas eosinophilic infiltration was not significantly altered. Furthermore, we demonstrated that local administration of antibodies to interleukin-16 also decreased airway hyperresponsiveness without affecting eosinophil infiltration, which is in agreement with results of previous experiments in which antibodies to interleukin-16 were administered intraperitoneally (Hessel et al., 1998). Peptides 1 and 2 were based on different C-terminal-located regions of the predicted amino acid sequence of recombinant human interleukin-16. These peptides have been demonstrated not to interfere with interleukin-16-induced chemotaxis in vitro in this study or with anti CD4 antibody binding to CD4 in another study (Keane et al., 1998). Therefore, we used these two peptides as control peptides.

Recently, it has been observed that in antigen-exposed asthmatics, as well as in our murine model of allergic asthma, interleukin-16 expression in epithelial cells and the presence of bioactive interleukin-16 in bronchoalveolar lavage fluid are up-regulated (Laberge et al., 1997; Hessel et al., 1998). Interleukin-16 is a natural soluble ligand for CD4, which activates several CD4-associated second messengers and induces chemotaxis of $CD4^+$ cells, including T lymphocytes, monocytes and eosinophils in vitro (Center et al., 1995, 1996). So far, no evidence exists for the induction of chemotaxis of $CD4^+$ cells in vivo. The amino acid sequence of peptide 3 is based on a hydrophilic C-terminal region of recombinant human interleukin-16. This region of recombinant human interleukin-16 is very likely to be exposed on the surface of the molecule (Keane et al., 1998). This particular peptide was capable of inhibiting recombinant murine interleukin-16-induced chemotaxis of mouse splenocytes in vitro. This is in agreement with

results of previous experiments in which it was demonstrated that peptide 3 is capable of decreasing recombinant human interleukin-16-induced chemotaxis of human $CD4^+$ cells (Keane et al., 1998). Furthermore, peptide 3 can partially prevent the binding of monoclonal antibodies to CD4 (Keane et al., 1998). These data, together with the observation that interleukin-16 uses CD4 as receptor, suggest that the C-terminal hydrophilic region of interleukin-16 is involved in binding of CD4. Furthermore, these data suggest that peptide 3 interacts with CD4, thereby inhibiting cross-linking by interleukin-16 and subsequent signalling. Recently, it has been observed that intraperitoneal administration of antibodies to interleukin-16 can partially inhibit airway hyperresponsiveness, whereas eosinophilia is not affected (Hessel et al., 1998). In the present study, it was demonstrated that inhibition of interleukin-16 interaction with CD4 by peptide 3 has effects similar to those of inhibition of interleukin-16-induced effects by intraperitoneal administration of anti-interleukin-16. Furthermore, as now described, local administration of anti-interleukin-16 also largely inhibits airway hyperresponsiveness without affecting eosinophil infiltration.

Recently, it has been demonstrated that interferon-γ plays an important role in the induction of airway hyperresponsiveness in our murine model since administration of antibodies to interferon-γ can inhibit the development of airway hyperresponsiveness without affecting eosinophil numbers in bronchoalveolar lavage fluid (Hessel et al., 1997). We can thus conclude that airway hyperresponsiveness and eosinophilia are not causally related in this model, which is consistent with results from another murine model of allergic asthma (Corry et al., 1996).

Production of interferon-γ by human peripheral blood mononuclear cells can be detected after incubation in vitro with a combination of interleukin-16 and interleukin-2 (Parada et al., 1998). Therefore, if peptide 3 inhibits cross-linking of CD4 molecules by interleukin-16 and subsequent local interferon-γ production, it could be speculated that airway hyperresponsiveness is decreased, whereas eosinophilia is not affected. The same could be true for the effects of antibodies to interleukin-16. In contrast, others have demonstrated that interferon-γ is a potent down-regulator of the development of airway hyperresponsiveness in different animal models (Lack et al., 1996; Nagai et al., 1997). Therefore, at present, no definite conclusions can be drawn as to how interleukin-16 plays a role in development of airway hyperresponsiveness in this murine model. Furthermore, if peptide 3 decreases airway hyperresponsiveness via prevention of interleukin-16-CD4 interaction, it can also not be excluded that peptide 3 has direct effects on other $CD4^+$ cells, e.g., monocytes or dendritic cells.

In the present study, airway eosinophilia was not inhibited by intra-airway treatment with either anti-interleukin-16 or peptide 3. Endogenous interleukin-16 could be important for the induction of airway hyperresponsiveness via recruitment of $CD4^+$ cells, including eosinophils. However, intraperitoneal or local administration of antibodies to interleukin-16 did not affect eosinophilia in this or other studies (Hessel et al., 1998). From previous studies, it is known that murine eosinophils do not express CD4 on their membrane as determined by FACS analysis (E.M. Hessel, personal communication). Furthermore, it has been reported that interleukin-16 does not prime or activate eosinophils in vitro (Rand et al., 1991). Finally, in vivo chemotaxis of $CD4^+$ cells, including eosinophils, by interleukin-16 could not be detected (J.J. de Bie, unpublished observations) and therefore inhibition of chemotaxis of eosinophils or lymphocytes by peptide 3 or the antibodies to interleukin-16 is not very likely.

Previously, it was demonstrated that inhibition of endogenously produced interleukin-16 by intraperitoneal treatment with anti-interleukin-16 inhibits production of antigen-specific IgE (Hessel et al., 1998). In agreement with this, local administration of anti-interleukin-16 also inhibited the up-regulation of antigen-specific IgE levels in serum after ovalbumin challenge (data not shown). However, no effects of peptide 3 on IgE production were observed (data not shown) and, therefore, it could be speculated that peptide 3 has lower stability or a shorter half-life than do antibodies to interleukin-16. However, further research is necessary to explain why antibodies to interleukin-16 do inhibit IgE production whereas peptide 3 does not.

In conclusion, our data suggest that peptide 3, which is based on the predicted amino-acid sequence of interleukin-16, can significantly inhibit airway hyperresponsiveness, whereas eosinophilia is not affected. Our results also showed that treatment with peptide 3 has the same effects as antibodies to interleukin-16, possibly via inhibition of the interaction between interleukin-16 and its receptor CD4. Therefore, peptide 3 could be useful as a lead compound in attempts to limit airway hyperresponsiveness via binding to CD4.

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