

## Rapid publications

## The IL-5 receptor on human bronchus selectively primes for hyperresponsiveness

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**Background:** The role of IL-5-induced eosinophilia in airway hyperresponsiveness has been questioned. In addition, eosinophil-independent IL-5-induced airway hyperresponsiveness has been demonstrated in animals.

**Objective:** In this study, IL-5 was investigated for direct effects on human bronchial responsiveness.

**Methods:** Human muscle preparations were isolated from organ donor and surgical tissue. Bronchus, jejunum, and saphenous vein were incubated for 24 hours in vitro with recombinant human (rh) IL-5. Contractility to acetylcholine (bronchus, jejunum) and phenylephrine (saphenous vein) was then investigated. RT-PCR was used to evaluate IL-5 receptor  $\alpha$  (IL-5R $\alpha$ ) expression in various tissues and to assess bronchus and saphenous vein eosinophils through use of CCR3 expression.

**Results:** rhIL-5 primed bronchus for an exaggerated contraction to acetylcholine. The acetylcholine concentration that produced 50% of the control maximum response was reduced 17- to 20-fold in bronchus treated with 1 and 10 nmol/L rhIL-5. The lower concentration of 0.1 nmol/L rhIL-5 had no effect. The rhIL-5 effect on bronchial contractility was attenuated by antibodies to IL-5 (TRFK-5; 100 nmol/L) and human IL-5R $\alpha$  (100 nmol/L). rhIL-5 (10 nmol/L) did not enhance contractility of saphenous vein or jejunum. When RT-PCR was used, IL-5R $\alpha$  expression was strong in bronchus muscle, weak in trachealis, saphenous vein, and atrial muscle, and undetectable in jejunum, urinary bladder, and pulmonary and renal artery muscle. Comparable weak expression of CCR3 was identified in bronchus and saphenous vein.

**Conclusion:** The findings are consistent with an airway tissue-selective expression of the IL-5 receptor that mediates IL-5-induced airway hyperresponsiveness independent of eosinophils. In asthma, in which IL-5 expression is elevated, IL-5 might directly induce bronchial hyperresponsiveness. (*J Allergy Clin Immunol* 2002;109:404-9.)

**Key words:** IL-5, acetylcholine, contractility, airway hyperreactivity, asthma, human bronchus, human saphenous vein, human jejunum, TRFK-5, anti-human IL-5R $\alpha$  antibody

## Abbreviations used

|                       |                                                                 |
|-----------------------|-----------------------------------------------------------------|
| ACh:                  | Acetylcholine                                                   |
| anti-IL-5R $\alpha$ : | Anti-human IL-5 receptor $\alpha$ subunit neutralizing antibody |
| EC <sub>50</sub> :    | Agonist concentration that induces a half-maximal response      |
| G3PDH:                | Glycerol 3-phosphate dehydrogenase                              |
| HSV:                  | Human saphenous vein                                            |
| KCl:                  | Potassium chloride                                              |
| pD <sub>2</sub> :     | -log EC <sub>50</sub>                                           |
| PE:                   | Phenylephrine                                                   |
| rh:                   | Recombinant human                                               |
| SMBM:                 | Smooth muscle basal medium                                      |
| TRFK-5:               | Anti-IL-5 mAb                                                   |

Asthma is a disease characterized by predominantly eosinophilic inflammation, airway obstruction, and non-specific hyperreactivity of the airways.<sup>1,2</sup> In human beings, the effect of IL-5 on immune function shows great specificity. Among inflammatory cells, IL-5 promotes maturation, growth, activation, and survival of human eosinophils and basophils exclusively.<sup>2-4</sup> Allergic IL-5<sup>-/-</sup> knockout mice lacking the IL-5 gene but showing normal T- and B-cell development and normal baseline eosinophils and immunoglobulin levels<sup>1</sup> do not develop pulmonary eosinophilia or airway hyperreactivity to antigen challenge, presumably because of avoidance of toxic eosinophilic granule product release in the airways.<sup>1,4,5</sup> These findings support efforts that target the IL-5-driven eosinophil inflammatory cascade with an anti-IL-5 antibody as a potential novel anti-inflammatory approach to asthma.<sup>1-6</sup>

Hakonarson et al<sup>7,8</sup> found that IgE complexed with rabbit trachea and human bronchus airway smooth muscle low-affinity IgE receptor in vitro induced a proasthmatic muscle phenotype characterized by upregulated IL-5, IL-1 $\beta$ , and G $\alpha$ i and, as shown in rabbit trachea, cholinergic hyperreactivity. These actions were inhibited by anti-IL-5 receptor  $\alpha$  (R $\alpha$ ) antibody.<sup>7,8</sup> Indeed, IL-5 upregulates IL-1 $\beta$  production by cultured human airway smooth muscle cells,<sup>8</sup> and IL-1 $\beta$  has been shown to increase intracellular Ca<sup>2+</sup> responses to contractile agonists<sup>9</sup> as well as desensitize the  $\beta_2$ -adrenoceptor-G $\alpha$ s-adenylate cyclase pathway<sup>10</sup> in human airway smooth

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muscle. It is thus plausible that as suggested by Hakonarson et al,<sup>8</sup> IL-5 might act directly on airway smooth muscle to induce hyperreactivity. Nevertheless, as of this writing, the effect of IL-5 on isolated human airway contractile responsiveness has not been demonstrated.

In the present experiments, we used isolated human bronchus, rhIL-5, and antibodies to both IL-5 and its receptor to evaluate the effects of exogenous IL-5 on human bronchus contractility to acetylcholine (ACh). In separate experiments, isolated human saphenous vein (HSV) and jejunum longitudinal smooth muscle preparations were incubated with rhIL-5 and contracted with phenylephrine (PE) and ACh, respectively, to investigate IL-5's effects on nonpulmonary smooth muscle systems. In addition, native expression of IL-5R $\alpha$  message was studied in these tissues as well as in various other human muscle-containing tissues. The present findings, though preliminary (because of limited patient samples), might have important implications for the mechanisms underlying airway hyperreactivity in asthma.

## METHODS

Donor lungs were procured by Tissue Transformation Technologies (Edison, NJ)/the International Institute for the Advancement of Medicine (Scranton, Pa) and the Anatomic Gift Foundation (Laurel, Md), shipped on wet ice in physiologic media, and received 15 to 44 hours after removal. Donor characteristics were as follows: 14 male and 9 female; mean age,  $39 \pm 4$  years (age range, 17-65 years). Relevant current medical-social histories included information on cigarette smoking (13 smokers and 10 nonsmokers), crack cocaine use (1), marijuana smoking (3), and bronchitis (1). The possibility of undocumented inflammation in the donor lungs as a result of smoking is an example of the kind of uncontrolled variable that might confound aspects of our results, and it underscores the preliminary nature of these studies. For the tension studies, transverse bronchus muscle strips (4 mm wide  $\times$  4-7 mm long) were isolated from bronchi that measured 7 to 12 mm in internal diameter. For later RT-PCR analysis, 5 mm segments of bronchus that measured 3 mm in internal diameter were cryopreserved. Bronchus was isolated immediately on arrival and used fresh or cryopreserved for later use.

Discard HSV obtained from 8 male and 4 female patients who had undergone coronary artery bypass graft procedures (mean age,  $66 \pm 3$  years; range, 51-83 years) and discard mid-distal jejunum obtained from 3 male and 7 female patients who had undergone intestinal bypass procedures (mean age,  $45 \pm 4$ ; range, 26-68 years) were supplied by Morristown Memorial Hospital (Morristown, NJ). Both tissues were stored and shipped as described previously for HSV<sup>11</sup> and received 24 to 48 hours after removal. HSV rings (4-5 mm in diameter  $\times$  5 mm long) were isolated immediately on arrival and cryopreserved for later use in the tension and RT-PCR investigations. Longitudinal jejunum strips (0.3 cm  $\times$  1.5 cm) were similarly isolated and cryopreserved.

Segments of a series of additional human tissues were isolated immediately on arrival (24-40 hours after removal) and cryopreserved for RT-PCR analysis only. Organ donor lungs, heart, and kidney procured by Tissue Transformation Technologies/the International Institute for the Advancement of Medicine served as the source of the trachealis muscle (female; age, 56 years), intrapulmonary artery ring (male; age, 54 years), right atrial muscle (female; age 49 years), and external renal artery ring (male; age 60 years) preparations. Discard muscular urinary bladder wall derived from a patient who had undergone cancer resection surgery (female;

age, 64 years; adjacent macroscopically normal tissue) was procured by the Cooperative Human Tissue Network (CHTN Western Division, Cleveland, Ohio). All donor organs and discard tissue were shipped in chilled physiologic solution on wet ice.

Cryopreservation and thawing of the tissues was performed as previously described for HSV,<sup>11</sup> except that 0.1 mol/L sucrose was omitted from the cryopreservation medium used for nonvascular tissues and the cryopreserved bronchus, trachealis, and urinary bladder wall segments were stored in liquid nitrogen-containing canisters.

For tension studies, bronchus, HSV, and jejunum (epithelium-, endothelium-, and mucosa-denuded, respectively) were incubated for 24 hours through use of 6-well polystyrene plates (Becton Dickinson, Franklin Lakes, NJ) containing 5.0 mL/well smooth muscle basal medium (SMBM) with rhIL-5, anti-IL-5 mAb (TRFK-5), or anti-human IL-5R $\alpha$  antibody (anti-IL-5R $\alpha$ ), alone or in combination, and penicillin-streptomycin solution (1:100 dilution). The incubation was performed in a Dubnoff incubator (25°C; shaking frequency, 33/min; atmosphere, 95% O<sub>2</sub>-5% CO<sub>2</sub>; Precision/Napco, Winchester, Va). The bronchus, HSV, or jejunum was then transferred to an organ bath containing 37°C Krebs buffer aerated with 95% O<sub>2</sub>-5% CO<sub>2</sub> (organ baths, buffer composition, and continuous isometric tension recording were as previously described<sup>11</sup>). A 1- to 2-hour equilibration at 2 g (bronchus), 1 g (HSV), or 0.5 g (jejunum) of initial tension preceded contractile concentration-response curves constructed through use of rising cumulative additions of ACh (0.1  $\mu$ mol/L to 1.0 mmol/L for bronchus) and PE (0.3  $\mu$ mol/L to 1 mmol/L for HSV) or noncumulative additions of ACh (0.1  $\mu$ mol/L to 1.0 mmol/L for jejunum). Separate maximal tension responses were then obtained to 1 mmol/L carbachol in bronchus or 80 mmol/L potassium chloride (KCl) in HSV. All tissue types were blotted dry and weighed at the end of the experiments. Estimated increase in tissue tension (in grams) over baseline tone was normalized as milligrams of tension per milligram of weight of the bronchus, HSV, and longitudinal jejunum smooth muscle preparations. Tissues that did not respond to the maximum tested concentrations of ACh (bronchus, jejunum) or PE and KCl (HSV) were not used in the analysis. Results were represented as the means  $\pm$  SEMs of patient responses calculated through use of replicate means of an individual patient's tissue responses. The agonist concentration causing a half-maximal response (EC<sub>50</sub>) was calculated through use of linear regression analysis of the concentration response curves and converted to the pD<sub>2</sub> (-log EC<sub>50</sub>). A 2-tailed paired *t* test was used for statistical analysis of paired patient data.

For RT-PCR analysis of IL-5R $\alpha$  mRNA, total RNA was isolated from cryopreserved and thawed human bronchus, trachealis, saphenous vein, renal and pulmonary arteries, jejunum, urinary bladder wall, and atria by means of the RNeasy Mini kit (Qiagen, Santa Clarita, Calif), used according to the manufacturer's instructions (including DNase digestion). All specimens except atria were denuded of epithelium, endothelium, or mucosa, as appropriate. Primers were designed to detect human IL-5R $\alpha$ , CCR3, and glyceral 3-phosphate dehydrogenase (G3PDH). Forward and reverse primer sequences used, respectively, were 5'CAGAACGACCACTCACTACTGG3' and 5'TCTCTGCTGTGACATTCAATGG3' for IL-5R $\alpha$ , 5'CCTTCTCTCTTCCTATCAATCC3' and 5'TGTGGATGGAGACAGAGC3' for CCR3, and 5'TCTCTCTCCGACTTCAACAGC3' and 5'CCTCTTCAAGGGGTC-TACATGG3' for G3PDH. Superscript One-Step RT-PCR system (GibcoBRL, Bethesda, Md) analysis was performed according to the manufacturer's instructions. The PCR reaction used 35 cycles at an annealing temperature of 55°C. RT-PCR DNA products were separated on 2% agarose gels and stained with ethidium bromide.

SMBM was purchased from Clonetics (BioWhittaker, Walkersville, Md). The rhIL-5 and TRFK-5 antibody stocks were prepared by Schering-Plough (Union, NJ). The anti-human IL-5R $\alpha$  neutralizing antibody was purchased from R&D Systems (Min-

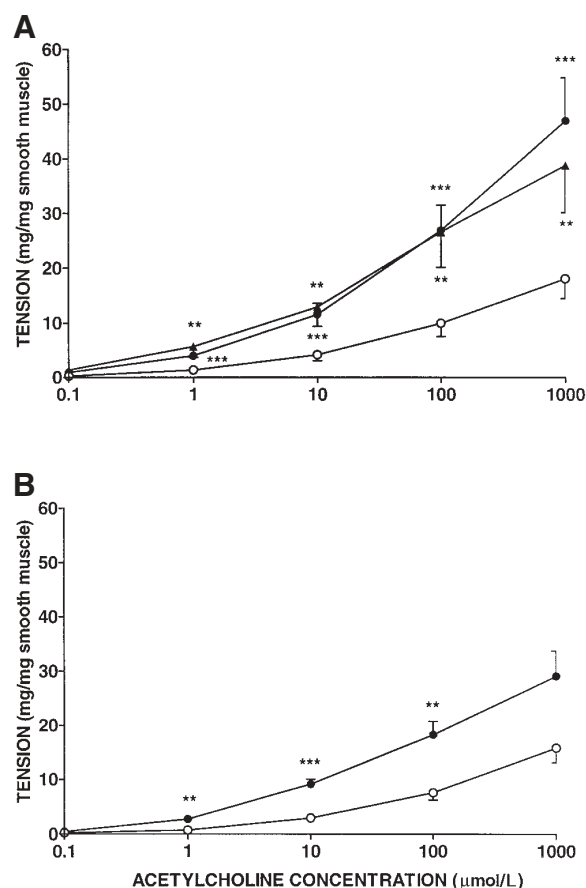


**FIG 1.** Paired control (open circles) and IL-5-treated (1 nmol/L, solid triangles; 10 nmol/L, solid circles) fresh (A) and cryopreserved (B) ( $n = 8$  and  $n = 15$ , respectively) human bronchus strip contractions to cumulative ACh. The symbols represent the means  $\pm$  SEMs of individual patient responses.  $**P \leq .05$  and  $***P \leq .01$ , different from the respective control group through use of a paired  $t$  test.

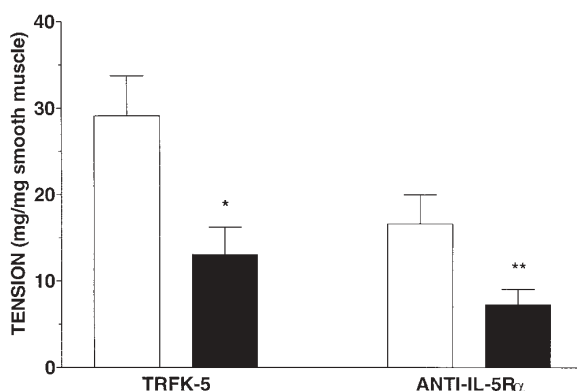


**FIG 2.** Paired cryopreserved human bronchus strip contractions to ACh (1 mmol/L) after incubation with IL-5 (10 nmol/L) in the absence (control; open bars) and presence (solid bars) of an anti-IL-5 antibody (TRFK-5, 100 nmol/L;  $n = 6$ ) or an anti-IL-5R $\alpha$  subunit antibody (100 nmol/L;  $n = 10$ ). Bars represent the means  $\pm$  SEMs of individual patient responses.  $*P \leq .06$  and  $**P \leq .05$ , different from the respective control group through use of a paired  $t$  test.

neapolis, Minn). Penicillin-streptomycin solution (100 $\times$ ), acetylcholine, carbachol, and PE were obtained from Sigma/RBI (St Louis, Mo). KCl was purchased from Fisher Scientific (Springfield, NJ). TRFK-5, rhIL-5, and anti-IL-5R $\alpha$  were diluted to final concentration in SMBM immediately before use. Concentrated aqueous stock solutions of ACh, carbachol, PE, and KCl were prepared fresh and diluted to final concentration in Krebs buffer.

## RESULTS

Control bronchus strips incubated for 24 hours in SMBM contracted in a dose-dependent fashion to ACh (Fig 1). The values for maximum contraction to carbachol (1 mmol/L) were  $26.4 \pm 6.0$  and  $26.7 \pm 4.2$  mg tension/mg smooth muscle, respectively, for fresh ( $n = 8$ ) and cryopreserved ( $n = 15$ ) tissue (data not shown). Both fresh (Fig 1, A) and cryopreserved (Fig 1, B) bronchus contractile responses to ACh were significantly enhanced after incubation with rhIL-5 (10 nmol/L). The IL-5-treated tissues demonstrated a  $>17$ -fold decrease in the ACh concentration needed to induce contraction equal to 50% of the control carbachol maximum (Fig 1). IL-5 (10 nmol/L) also augmented the carbachol maximum contraction obtained in fresh ( $66.9 \pm 11.8$  mg tension/mg smooth muscle;  $n = 8$ ;  $P = .01$ ) and cryopreserved ( $35.0 \pm 6.9$  mg tension/mg smooth muscle;  $n = 15$ ) bronchus (data not shown). Incubation with 1 and 10 nmol/L IL-5 induced similar augmentation of bronchial contractility to ACh (Fig 1, A). No significant effect on bronchial reactivity was seen after incubation with 0.1 nmol/L IL-5 (eg, respective control and treated paired patient responses to 1 mmol/L ACh were  $16.8 \pm 3.1$  and  $32.6 \pm 9.9$  mg tension/mg smooth muscle;  $n = 7$ ; data not shown). The rhIL-5 (10 nmol/L) enhancement of cryopreserved bronchus contractility to ACh was inhibited by co-incubation with either TRFK-5 (100 nmol/L; Fig 2) or anti-human IL-5R $\alpha$  (100 nmol/L; Fig 2). Control incubations with either TRFK-5 or anti-IL-5R $\alpha$  (each at 100 nmol/L) alone had no significant effect on contractions to ACh ( $n = 6$  and  $n = 10$ , respectively; data not shown).

PE induced dose-dependent contractions in cryopreserved HSV after the 24-hour incubation in SMBM (Table I). The mean PE maximum was 71% of the response to 80 mmol/L KCl ( $9.8 \pm 2.1$  mg tension/mg smooth muscle;  $n = 9$ ). In contrast to human bronchus, incubation of HSV with rhIL-5 (10 nmol/L) altered neither the sensitivity to PE nor the magnitude of the contractions to PE (Table I) and KCl ( $9.6 \pm 2.0$  mg tension/mg smooth muscle;  $n = 9$ ). Similarly to what was seen with HSV, agonist-induced dose-dependent contractions occurred in cryopreserved human jejunum longitudinal muscle after 24 hours of incubation in SMBM, and contractility to ACh was not enhanced by treatment with rhIL-5 (10 nmol/L; Table I).

RT-PCR analysis of IL-5R $\alpha$  gene expression was performed on human preparations that included bronchus ring, HSV, and jejunum, each taken from 4 patients. Bronchus and jejunum samples included 4 of the patients used in the contractility studies; HSV samples included 1 of the patients used in the contractility studies. Single-

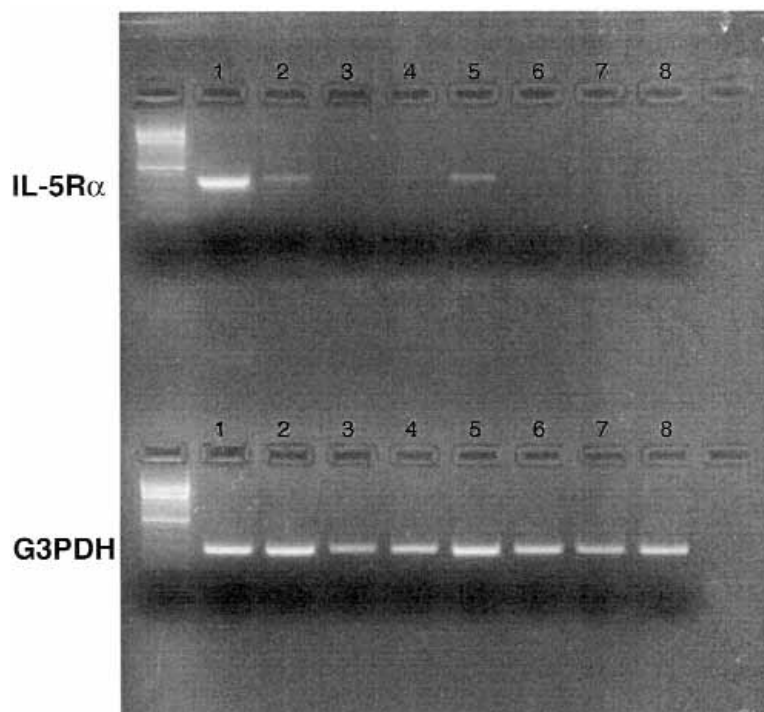


**FIG 3.** RT-PCR analysis of human IL-5R $\alpha$  and G3PDH gene expression in human bronchus (*lane 1*), saphenous vein (*lane 2*), urinary bladder wall (*lane 3*), trachea (*lane 4*), atria (*lane 5*), renal artery (*lane 6*), pulmonary artery (*lane 7*), and jejunum (*lane 8*). The unlabeled lane contains size marker DNA, and the RT-PCR-generated IL-5R $\alpha$  and G3PDH DNA are 438 and 302 base pairs, respectively.

**TABLE I.** Contractions of paired control and IL-5-treated (10 nmol/L) cryopreserved human saphenous vein ring and human jejunum longitudinal strip preparations to phenylephrine and acetylcholine, respectively

| Tissue  | No. of patients | Maximum agonist response* (mg/mg smooth muscle) |            | Agonist pD <sub>2</sub> † |      |
|---------|-----------------|-------------------------------------------------|------------|---------------------------|------|
|         |                 | Control                                         | IL-5       | Control                   | IL-5 |
| HSV     | 9               | 6.9 ± 1.5                                       | 7.0 ± 1.4  | 5.7                       | 5.7  |
| Jejunum | 10              | 14.8 ± 4.2                                      | 12.9 ± 3.7 | 5.3                       | 5.3  |

HSV, Human saphenous vein.

\*Expressed as the means ± SEMs of individual patient tension responses.

†The pD<sub>2</sub> (-log EC<sub>50</sub>) is estimated from the mean concentration response curve.

patient samples of trachealis, pulmonary and renal artery rings, urinary bladder wall, and right atria were also analyzed. The trachealis was derived from a patient who also supplied bronchus. Strong expression for IL-5R $\alpha$  was detected in bronchus (4/4), whereas weak expression ( $\leq 10\%$  relative to bronchus) was detected in trachealis, HSV (1/4), and atria; no expression was detected in the jejunum (4/4), urinary bladder, or pulmonary and renal artery preparations. G3PDH expression was uniformly strong in all of the human muscle preparations analyzed. A representative gel showing IL-5R $\alpha$  and G3PDH expression is displayed in Fig 3. In addition, RT-PCR analysis of CCR3 gene expression was performed on the RNA extracted from the 4 human bronchus and 4 HSV rings. Comparable weak CCR3 expression was detected in 4 of 4 samples from both tissue types (data not shown).

## DISCUSSION

We have demonstrated for the first time that IL-5 elicits cholinergic hyperresponsiveness in isolated normal human bronchus smooth muscle. The present investigations also demonstrate that the augmentation of cholinergic contractility induced by rhIL-5 is blocked by neutralizing anti-IL-5 and anti-human IL-5R antibodies. Evidence is thus provided that IL-5 ligand-receptor interaction mediates the development of enhanced human bronchial contractility in the absence of eosinophil influx. These findings are consistent with the observations of Hakonarson et al<sup>8</sup> showing direct induction by IL-5 of augmented cholinergic contractility in isolated rabbit airway smooth muscle. In further support of this concept, IL-5 and IL-4, but not eosinophils or IgE, were

critical for the development of allergen-induced airway cholinergic hyperresponsiveness in a human T-cell-SCID mouse chimera model of asthma.<sup>12</sup> That activated T cells might also play a role in human airway disease is suggested by their ability to bind human airway smooth muscle and alter its HLA-DR and adhesion molecule expression.<sup>13</sup>

There is a large body of evidence that IL-5 is obligatory for the development of the prominent eosinophilic lung inflammation known to correlate with increased airway responsiveness in asthma.<sup>1,4</sup> Studies sampling both allergic and intrinsic asthma patient populations have demonstrated elevated IL-5 in the bronchoalveolar lavage fluid.<sup>14</sup> Furthermore, the only interleukin-effector cell pathway that is common to both allergic and intrinsic asthma and that has been related to symptom severity is IL-5-mediated eosinophil activation.<sup>14</sup> The proasthmatic activity of IL-5 and its selective tropic and trophic actions on human lung eosinophils and basophils among immune competent cell types makes IL-5 an attractive therapeutic target, because immunosuppressive consequences of therapy should be minimal.<sup>4</sup>

The finding that an IL-5 ligand-receptor interaction on airway smooth muscle can mediate hyperresponsiveness independently of eosinophil-induced tissue damage might have important implications for an understanding of the mechanisms underlying airway hyperreactivity in asthma and for the design of therapeutic regimens that target IL-5. This finding suggests that in addition to reducing eosinophilic inflammation in the airways, anti-IL-5 therapy might reduce the hyperreactivity of asthmatic airways and improve lung function. It should be noted, however, that larger doses of mAbs to IL-5 were needed to block airway hyperreactivity than to block eosinophil infiltration into lung tissue and bronchoalveolar lavage fluid in antigen-challenged allergic guinea pigs.<sup>4,6</sup> In limited human trials, single-dose administration to asthmatic individuals of the humanized anti-IL-5 mAbs SCH 55700 and SB-240563 reduced peripheral blood eosinophil counts for  $\geq 90$  days. However, SCH 55700 produced a small improvement in FEV<sub>1</sub>, and SB-240563 had no effect on the bronchial responsiveness to histamine.<sup>15,16</sup> The present finding that IL-5 induces human bronchial hyperreactivity in the absence of eosinophil influx, combined with the human and guinea pig anti-IL-5 antibody results, suggests a possible divergence between IL-5-induced eosinophilia and airway hyperreactivity. Accordingly, effective dosing regimens for asthmatic IL-5-dependent eosinophilia and hyperreactivity might differ. In addition, an evaluation of the limitations of the SB-240563 trial<sup>17</sup> raised various concerns, including questions about whether the statistical power was insufficient (because of the number of subjects) and the use of a parallel design, which weakens confidence in the lack of treatment effect on allergen-induced change in airway histamine responsiveness. The SCH 55700 trial also exhibits such limitations as small patient populations treated with high-dose steroids, parallel design, and use of only FEV<sub>1</sub> to evaluate airway responsiveness. Further-

more, these trials were single-dose studies; additional investigations are needed to effectively evaluate the role of IL-5 in asthma and, more specifically, in the bronchial hyperresponsiveness seen in this disease.

In additional studies conducted under conditions equivalent to those used in these cryopreserved bronchus investigations, IL-5 failed to enhance either cryopreserved HSV smooth muscle contractility to PE (adrenergic response) or cryopreserved jejunum longitudinal smooth muscle contractility to ACh (cholinergic response). These findings indicate that the effects of IL-5 might selectively induce airway smooth muscle hyperresponsiveness. The eotaxin receptor CCR3 is constitutively and strongly expressed on eosinophils.<sup>18,19</sup> The present RT-PCR finding of comparable low CCR3 expression in bronchus and HSV suggests that the restriction to airway of IL-5 responsiveness does not involve a selective presence of numerous eosinophils in isolated human bronchus. Furthermore, uniformly strong expression of the housekeeping gene G3PDH in all of the sampled tissues provides assurance that general RNA degradation cannot explain the CCR3 results and the observed differential expression of IL-5R $\alpha$  message. The restriction of IL-5-induced hyperresponsiveness to the airway thus appears to be due to tissue-specific expression of the IL-5 receptor.

IL-5 augmented human bronchus contractility similarly at concentrations of 1 and 10 nmol/L, whereas the no-effect level for IL-5 in the present study was 0.1 nmol/L. The dissociation constant of the human eosinophil IL-5 receptor is 0.2 to 0.3 nmol/L.<sup>4</sup> Thus, concentrations of IL-5 very similar to its reported dissociation constant can augment isolated human bronchus contractility.

The tissue response variance in general—and the specific variance with regard to IL-5 treatment in the present tension studies—might be related to heterogeneity of the donor population (ie, age, sex, environment, and genetic and medical-social history) and tissue-handling variables. Although the utility of cryopreserved bronchus and HSV for study of cholinergic<sup>20</sup> and adrenergic<sup>11,21</sup> contractility has been previously demonstrated, cryopreservation might account for the relative decrease in responsiveness to ACh that was noted in the experiments in which bronchus was used. Little is known about the functional responsiveness of human jejunum after prolonged cold storage followed by cryopreservation at ultra-low temperatures. Others have indicated that prolonged (18-hour) storage of human jejunum and ileum longitudinal muscle preparations in physiologic media at 4°C has minimal effects on functional muscarinic ACh<sup>22</sup> and other pharmacologic sensitivities.<sup>23</sup> However, transplantation-related experiments in which rat intestine was used (eg, those conducted by Sakai et al<sup>24</sup>) suggest that this organ's viability might be adversely affected by cold storage beyond 24 hours. Rat transplantation experiments have also shown ultra-low temperature cryopreservation to be useful for the storage of intestine, inasmuch as this organ remained viable for transplant after 30 days of cryopreservation at -180°C.<sup>25</sup> Nevertheless, ACh pro-

duced dose-dependent isometric contractions in most of the cryopreserved human jejunum longitudinal preparations used in the present investigations, and its apparent potency was comparable to that obtained by other investigators who used isometric contractions of freshly isolated human ileum and jejunum longitudinal strips ( $pD_2 \equiv 6.2$ ).<sup>26</sup>

In conclusion, IL-5 can selectively and directly induce bronchial hyperreactivity in human airway smooth muscle. The present findings and those of Hakonarson et al<sup>7,8</sup> provide added impetus to the development of anti-IL-5 therapy for asthma.

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