Identification of R146225 as a Novel, Orally Active Inhibitor of Interleukin-5 Biosynthesis

JEAN VAN WAUWE, FRANS AERTS, MARINA COOLS, FREDERIK DEROOSE, EDDY FREYNE, JAN GOOSSENS, BART HERMANS, JEAN LACRAMPE, HEIDI VAN GENECHTEN, FRANS VAN GERVEN, and GRETA VAN NYEN

Janssen Research Foundation, Beerse, Belgium

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ABSTRACT

Interleukin (IL)-5 regulates the growth, differentiation, and activation of eosinophils. When activated, eosinophils release an array of proinflammatory and cytotoxic products and act as prominent effector cells in the process of allergic inflammation. Depriving eosinophils of IL-5 may therefore represent a viable approach to treat allergic disorders. This study describes the identification of R146225, a novel six-substituted azauracil derivative, as a potent, orally active inhibitor of IL-5 biosynthesis, capable of reducing pulmonary eosinophilia in mice. In vitro, R146225 inhibited IL-5 protein formation by activated human whole blood (IC_{50} = 34 nM), human peripheral blood mononuclear cells (IC_{50} = 24 nM), and murine spleen cells (IC_{50} = 6 nM). In contrast, the compound enhanced generation of interferon-γ and had little or no inhibitory effect on the production of IL-2 and IL-4. Reverse transcription-polymerase chain reaction analysis of stimulated whole blood cells indicated R146225’s ability to down-regulate IL-5 mRNA expression. In vivo p.o. administration of R146225 (2.5 mg/kg) to mice before an i.v. anti-CD3 antibody challenge reduced IL-5 but enhanced interferon-γ serum levels, without affecting IL-2 and IL-4 production. Analogous to the in vitro results, R146225 suppressed splenic IL-5 mRNA expression, while message levels of the other cytokines remained unchanged. Moreover, p.o. dosing of R146225 (0.6–2.5 mg/kg) dose dependently reduced the pulmonary accumulation of eosinophils induced in mice by an intranasal instillation of Cryptococcus neoformans. Based on these data, R146225 may be useful in the therapy of eosinophil-driven allergic conditions.

Increased accumulation of eosinophils in blood and inflamed tissues is a characteristic feature of the major allergic diseases, viz., asthma, rhinitis, and atopic dermatitis (Costa et al., 1997). The ability of eosinophils to synthesize and release a series of proinflammatory and cytotoxic products, such as basic granule proteins, proteases, sensory neuromedullins, leukotrienes, oxygen radicals, and cytokines, has incriminated these polymorphonuclear leukocytes as principal effector cells in the inflammatory process of allergy (Giembycz and Lindsay, 1999). For their growth, differentiation, activation, and survival, eosinophils are dependent on the availability of T-cell-derived hematopoietic cytokines, such as granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5 (Humbert, 1996). Of these proteins, IL-5 stands out for its nonredundant and eosinophil-dedicated activities (Egan et al., 1996; Hogan and Foster, 1996; Sehmi and Denburg, 1999). In bone marrow, IL-5 is the sole cytokine that regulates the final step of eosinophil differentiation and controls the entry of matured eosinophils to the bloodstream. In blood, IL-5 primes blood-borne eosinophils for adherence to and transmigration through the vascular endothelium. In tissues, IL-5 acts as a survival factor for eosinophils by delaying their apoptotic death. Numerous studies using IL-5 transgenic mice or animals treated with neutralizing anti-IL-5 antibodies have corroborated the key role of IL-5 in the process of eosinophilia, inflammation, and/or tissue alterations in response to an allergic challenge (Egan et al., 1996; Hogan and Foster, 1997; Lee et al., 1997; Hamelmann and Gelfand, 1999). Moreover, clinical studies in humans have indicated an interdependency between the level of IL-5 mRNA/protein expression, eosinophilia, and/or allergic symptoms (Robinson et al., 1993; Egan et al., 1996; Humbert et al., 1997; Kimura et al., 1998; Masuyama et al., 1998). All in all, the central position of eosinophils in the process of allergic inflammation, together with the selective effect of IL-5 on eosinopoiesis, identifies this cytokine as a viable target for the treatment of asthma and allergic diseases (Hogan and Foster, 1997; Okudaira and Mori, 1998; Lalani et al., 1999). Among the several potential strategies to target IL-5 (Minnicozzi, 1999), we opted for the development of orally active inhibitors of IL-5 production. By compound library screening using the phytotermagglutinin (PHA)-stimulated human whole blood assay (Van Wauwe et al., 1995), we hit upon a series of six-substituted azauracil derivatives with...
micromolar IL-5-inhibiting potency. Subsequent targeted chemical synthesis resulted in R146225 or (A)-(−)-2-[3,5-dichloro-4-[(4-chlorophenyl)2-pyrimidinylthio)methyl]phenyl-1,2,4-triazine-3,5(2H,4H)dione, now identified as a potent, orally active inhibitor of IL-5 protein production and IL-5 mRNA expression. The chemical structure of R146225 is depicted in Fig. 1.

**Materials and Methods**

**Animals.** Female Balb/c and C57Bl/6 mice (20–22 g) were obtained from Ifa Credo (Brussels, Belgium) and Charles River (Sulzfeld, Germany), respectively. The animals were housed in an air conditioned room at 21°C (12-h light/dark cycle) and provided with food and water ad libitum.

**Chemicals.** R146225 was synthesized at the Department of Medicinal Chemistry, Janssen Research Foundation (Beerse, Belgium) according to methods described in patent application WO 9902504. For in vitro experiments, the compound was dissolved at 5 mM in dimethyl sulfoxide and appropriately diluted with culture medium so that the final dimethyl sulfoxide concentration was maintained at 0.2%. Culture medium consisted of RPMI 1640 supplemented with 2 mM glutamine, 100 U/ml penicillin, and 10 µg/ml streptomycin. PHA was obtained from Murex (Dartford, UK). The hamster anti-mouse CD3 antibody (anti-CD3) was isolated by protein A-Sepharose affinity chromatography from supernatants of clone 154-2C11 hybridoma cells, obtained from the American Type Culture Collection (Rockville, MD). Anti-human CD28 antibody (anti-CD28) was purchased from Serotec (Oxford, UK). For in vivo experiments, R146225 was suspended in a 1% solution of Tween 80 (Sigma, Bornem, Belgium) in water and orally administered to mice in a volume of 0.1 ml/10 g of body weight.

**In Vitro Cytokine Production.** Blood from adult healthy donors was drawn into heparinized syringes (12.5 U of heparin/ml), and cultured as described (Van Wauwe et al., 1995). Briefly, blood samples were 3-fold diluted in culture medium and 300-µl fractions were cultured with 100 µl of PHA (final concentration 5 µg/ml) and 100 µl of R146225 (final concentration 1–1000 nM). Human peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway) and suspended at a density of 2 × 10^6 cells/ml in culture medium containing 10% heat-inactivated fetal calf serum. PBMCs (100 µl) were cultured after addition of 50 µl of R146225 (final concentration 1–1000 nM) and 50 µl of a mixture of PHA (final concentration 5 µg/ml) and anti-CD28 (final concentration 1 µg/ml). Mouse spleen cells were prepared as described (Cools et al., 1992) and suspended at a density of 6 × 10^6 cells/ml in culture medium containing 50 µM 2-mercaptoethanol (Sigma) and 5% heat-inactivated fetal calf serum (Life Technologies, Merelbeke, Belgium). Splenocytes (100 µl) were cultured together with 50 µl of R146225 (final concentration 1–1000 nM) and 50 µl of anti-CD3 (final concentration 5 µg/ml). All cultures were performed at 37°C in a humidified 6% CO2 atmosphere. At the specified times, cell supernatants were harvested for cytokine protein determinations and cellular RNA was isolated for cytokine mRNA measurements.

**In Vivo Cytokine Production.** Balb/c mice were treated orally with R146225 or vehicle (1% Tween 80) 1 h before an i.v. challenge with 0.2 ml of saline containing 2.5 µg of anti-CD3. At indicated times after the antibody challenge, blood samples were obtained and allowed to clot overnight at 4°C. Serum samples were then stored at −20°C until tested for the presence of cytokines. In some experiments, spleens were excised and homogenized in RNA lysis buffer to isolate cellular RNA.

**Cryptococcus neoformans-Induced Lung Eosinophilia.** Cryptococcus neoformans strain RV 11852 (ATCC 32265) was obtained from the American Type Culture Collection. For in vivo experiments, R146225 was administered once daily from day 3 up to 13 after infection. Preliminary experiments have indicated that R146225 treatment did not interfere with lung colonization by C. neoformans: CFU per lung, at day 14 post infection, were 5.7 ± 0.1 × 10^6 and 5.7 ± 0.1 × 10^6 in vehicle- or R146225 (10 mg/kg)-treated mice, respectively.

**Cytokine Measurements.** Cytokine protein concentrations were determined by sandwich ELISA as described (Van Wauwe et al., 1995). Murine monoclonals used as capture antibodies to human cytokines were obtained from R&D Systems (Abingdon, UK) and code named MAB 202, 204, 205, and 285 for IL-2, IL-4, IL-5, and IFN-γ, respectively. Biotinylated goat polyclonal antibodies used to detect human cytokines were from R&D Systems (BASF 202, 204, 205, and 285). Rat monoclonals to capture mouse cytokines were purchased from Pharmingen (Becton Dickinson, Erembodegem, Belgium) or mouse spleen in 1 ml of Ultra-Clean Buffer (Boehringer-Ingelheim, Germany). In some experiments, spleens were excised and homogenized in RNA lysis buffer to isolate cellular RNA.

**RNA Extraction and Reverse Transcription-PCR.** Cellular RNA was prepared by homogenizing pellets whole blood cells after lysis of erythrocytes with red blood cell lysis buffer (Boehringer-Mannheim, Brussels, Belgium) or mouse spleen in 1 ml of UltraSpec-II RNA lysis buffer, followed by RNA extraction according to the manufacturer’s instructions (Biotex Laboratories, Houston, TX). RNA content was determined by the absorbance measurement at 260 nm. Analysis for the presence of mRNAs was carried out using the Titan One Tube reverse transcription-PCR system (Roche Molecular Biochemicals, Brussels, Belgium). Briefly, 200 ng of RNA was reverse transcribed and amplified in 50 µl of reverse transcription-PCR buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 1.5 mM MgCl2), containing 0.2 mM dNTP, 5 mM dithiothreitol, 8 U of RNase inhib-

![Fig. 1. Chemical structure of R146225.](image-url)
itor, enzyme mix (AMV reverse transcription and Expand High Fidelity enzymes), and 0.4 μM 5′- and 3′-specific primers for the human or murine cytokine genes under consideration. To account for quantitative and/or qualitative differences in the RNA preparation, primers for the β-actin gene were used in each experiment. Samples were transferred to a thermocycler (PCT-200 Peltier thermal cyclor; MJ Research, Watertown, MA); incubated at 50°C for 30 min; and subjected to a denaturation step (94°C, 2 min) and to 26 to 32 cycles consisting of 30 s at 94°C, 1 min at 55°C, and 30 s at 72°C, followed by a final elongation step 4 min at 72°C. After amplification, 15 μl of PCR product was mixed with 5 μl of gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water), electrophoresed in a 2% agarose gel at 60 V, and visualized by UV light illumination after ethidium bromide staining and quantified using the Lumi-Imagen F1 Workstation (Roche Molecular Biochemi-
cals).

Specific primers were obtained from Biosource Europe (Nivelles, Belgium). Their sequences were as follows: human IL-2: 5′ primer, ATGTACAGGATGCAACTCCTGTCTT and 3′ primer, GTCACGTGTAGATGATGCTTTGAG; human IL-4: 5′ primer, ATGGGCTCACTCCTCCAAGTCTGGG and 3′ primer, GGGTCAGAAGGATTCCTATG and 3′ primer, CTCCCAACTGCTT and 3′ primer, CTCCCAACTGCTT and 3′ primer, GCCCAAGCAGTCTTGGC; human IL-5: 5′ primer, GCTTTGAGAAAGGGCTATCCA; human IL-12: 5′ primer, AAGCTTACACACTGCATCT- TG and 3′ primer, GACCTCAAAAGTCGTCATCT and 3′ primer, CTCAATCTAGAGTAATCCA; human IL-13: 5′ primer, AGTTATATCTTGGCTTTTCA and 3′ primer, CTCAGTACTACGAGTAATCCA; mouse IL-4: 5′ primer, GAACTCTTGCAAGGTA-
cals).

Data Analysis. Data are expressed as mean ± S.E. The 50% inhibition concentrations (IC_{50}) were calculated by probit analysis. Comparisons between two groups were performed by Student’s t test and among three or more groups by Dunnett’s test.

Results

Effect of R146225 on In Vitro IL-5 Protein Production. To determine the inhibiting activity of R146225 on IL-5 protein production, cultures of human whole blood, human PBMCs, and mouse splenocytes were preincubated for 30 min with or without R146225 (1–1000 nM), and stimulated by the addition of PHA, PHA + anti-CD28, or anti-CD3, respectively. At 48 h after stimulation, supernatant levels of IL-5 were assessed by ELISA. As shown in Fig. 2, R146225 potently inhibited the IL-5 production by stimulated cultures of human whole blood (IC_{50} = 34 ± 10 nM), human PBMCs (IC_{50} = 24 ± 8 nM), and mouse splenocytes (IC_{50} = 6 ± 2 nM).

Effects of R146225 on In Vitro IL-2, IL-4, and IFN-γ Protein Production. To determine the cytokine specificity of R146225, we compared its effects on the production of IL-2, IL-4, IL-5, and IFN-γ by activated human whole blood, human PBMCs, and mouse splenocyte cultures. Cells were activated in the absence or presence of R146225 (1 μM) and supernatants were recovered at the time of maximal cytokine production, i.e., 24 h for IL-2 and 48 h for IL-4, IL-5, and IFN-γ. As shown in Fig. 3, in conditions where R146225 markedly (65–85% inhibition) suppressed the IL-5 production, the compound had no or only partial effects on the biosynthesis of IL-2 (13–25% reduction) and IL-4 (8–30%). In contrast, in the presence of R146225, generation of IFN-γ by activated whole blood, PBMCs, and splenocyte cultures was enhanced by 12 ± 4, 20 ± 6, and 57 ± 8%, respectively.

Effect of R146225 on In Vitro Cytokine mRNA Expression. To assess the potential effects of R146225 on cytokine production at the mRNA level, PHA-stimulated or unstimulated whole blood was cultured for 4, 12, and 16 h.
with or without R146225 (1 μM). Cellular RNA was extracted and reverse transcription-PCR analyzed for mRNA levels of IL-2, IL-4, IL-5, IFN-γ, and the housekeeping gene β-actin. The results are shown in Fig. 4. Unstimulated whole blood cells did not contain detectable levels of cytokine mRNA, but PHA stimulation induced a rapid (6-h time point) and stable (12- and 24-h) expression of IL-2, IL-4, and IFN-γ transcripts. In contrast, IL-5 mRNA expression only gradually increased from a barely detectable (at 6 h) to abundant levels (at 24 h). At this latter time point, R146225 markedly (>80% inhibition) down-regulated the IL-5 message. Except for a modest inhibition (by ~30%) on IL-4 mRNA expression at 16 and 24 h, R146225 did not affect gene expression of IL-2, IFN-γ, or β-actin.

Effect of R146225 on IL-5 Protein Production In Vivo. To establish the in vivo IL-5-reducing activity of R146225, mice were p.o. treated with vehicle (1% Tween 80) or R146225 (2.5 mg/kg). One hour later, the animals were i.v. injected with anti-CD3 (2.5 μg/mouse). Two, 4, 6, 8, and 12 h later, serum IL-5 levels were measured by ELISA. As shown in Fig. 5, R146225 caused a partial (40–60%) inhibition of IL-5 production at 6, 8, and 12 h after antibody treatment, but was without effect at the 2- and 4-h time points. To determine the compound’s potency, mice were p.o. treated with vehicle or graded doses of R146225 (0.04–10 mg/kg) before (~1 h) an i.v. challenge with anti-CD3. Six hours later, the level of circulating IL-5 was determined. Figure 6 shows that R146225 pretreatment significantly reduced IL-5 production after administration of doses between 10 mg/kg (46 ± 9% inhibition) and 0.16 mg/kg (29 ± 9% inhibition).

Effect of R146225 on IL-2, IL-4, and IFN-γ Protein Production In Vivo. To determine the in vivo cytokine selectivity of the test compound, mice were p.o. treated with vehicle or R146225 (2.5 mg/kg) and, 1 h later, i.v. injected with anti-CD3. At the time of maximal serum production (2 h for IL-2, IL-4, and IFN-γ and 6 h for IL-5 as determined in preliminary time course experiments), serum levels of the cytokines were assessed (Fig. 7). Whereas R146225 significantly attenuated serum IL-5 levels by 47 ± 4%, it had no effect on the anti-CD3-induced serum levels of IL-2 and IL-4. However, R146225 virtually doubled (from 12,600 to 23,100 pg/ml) the serum production of IFN-γ.

Effect of R146225 on IL-2, IL-4, IL-5, and IFN-γ mRNA Expression In Vivo. To establish whether R146225 affected the in vivo cytokine mRNA levels, mice were p.o. treated with vehicle or R146225 (2.5 mg/kg), before (~1 h) an i.v. injection with anti-CD3. After another 1.5 or 5 h, spleens were excised, RNA was isolated, and the level of cytokine and β-actin mRNA was evaluated by reverse transcription-PCR. Figure 8 shows that treatment with R146225 strongly (>80% inhibition) suppressed the anti-CD3-induced splenic accumulation
Effect of R146225 on IL-2, IL-4, IL-5, and IFN-γ mRNA expression in mice. Vehicle (□) or R146225 (2.5 mg/kg) (□) were p.o. administered 1 h before an i.v. injection with 2.5 μg/ml anti-CD3 antibody. Blood was collected after another 2 h (for IL-2, IL-4, and IFN-γ) or 6 h (for IL-5) and serum levels of cytokines were measured. Each value represents the mean ± S.E. using six to eight animals per treatment group. Representative data from one of three independent experiments are shown. *P < .05 and **P < .01 versus vehicle-treated animals.

Discussion

Considering the prominent role that the eosinopoietic cytokine IL-5 plays in the pathogenesis of allergic disorders, we initiated a screening program to hit upon novel IL-5-inhibiting compounds. This effort eventually led to the identification of R146225 as an in vitro and in vivo inhibitor of IL-5 protein synthesis and mRNA expression, capable of reducing the pulmonary accumulation of eosinophils in C. neoformans-infected mice.

Our in vitro data indicate that R146225 acted as a potent inhibitor of IL-5 protein formation. Irrespective of the used cell system (human whole blood, human PBMCs, mouse splenocytes) or stimulating agent (PHA, PHA + anti-CD28 antibody, anti-CD3 antibody), submicromolar concentrations of R146225 suppressed by more than 70% the IL-5 protein levels in the cell supernatants. R146225 reduced only modestly (by less than 30%) the production of IL-2 and IL-4, but it enhanced IFN-γ protein formation, weakly (10–30% increase) in the human cell systems, but more strongly (50–65% enhancement) in mouse splenocyte cultures. This pattern of cytokine modulation clearly differentiates R146225 from other known IL-5-inhibiting compounds. For instance, the corticosteroid dexamethasone or the phosphodiesterase 4 inhibitor rolipram suppresses with comparable potency the production of IL-5, IL-2, and IFN-γ (Van Wauwe et al., 1995), whereas the T-cell immunosuppressants cyclosporin and FK506 down-regulate the production of IL-2, IL-4, and IFN-γ more strongly than that of IL-5 (Pacocha et al., 1999). Also the two recently described IL-5-inhibiting/IFN-γ-enhancing compounds PNU-142731A and M50367 are different from R146225 because they also suppress the production of IL-4, IL-6, and IgE (Chin et al., 1999; Kato et al., 1999). Curiously, for both R146225 and the above-mentioned anti-inflammatory compounds (Rolfe et al., 1992, 1997; Foissier et al., 1996; Barnette et al., 1998; Pacocha et al., 1999), inhibition of IL-5 protein production was preceded by a reduction of IL-5 mRNA expression. How R146225 reduces IL-5 mRNA expression is unclear. Acceleration of IL-5 mRNA degradation by R146225 appears unlikely: such action goes counter to the high stability of IL-5 transcripts (Umland et al., 1998) and, indeed, pulse-chase experiments using IL-5-producing activated murine EL-4 thymoma cells indicated that R146225 did not alter the IL-5 mRNA decay rate (J. Van Wauwe, unpublished data). Also because IL-5 protein synthesis is primarily regulated at the transcriptional level (Rolfe and Sewell, 1997), it appears more plausible that R146225 affects the process of IL-5 gene transcription. Recent data on the nuclear pathways regulating the human IL-5 gene have revealed the presence of multiple regulatory elements in the 5′-flanking region of this gene. This region contains positive regulatory elements, such as a conserved lymphokine element O, that interact with the transcription factors Oct1, Oct2, Ets1, and Ets2, and activator protein-1 members JunD and Fra-2 (Schwenger et al., 1999), a GATA-4 binding site (Yamagata et al., 1995), and a nuclear factor of activated T
cells binding site (De Boer et al., 1999), together with negative regulatory elements that bind the YY1, Oct1, and an Oct-like nuclear factor (Mordvinov et al., 1999) or a combination of YY1 and nuclear factor of activated T cells transcription factors (Schwenger et al., 1999). Experiments are now underway to determine the effect of the test compound on the human IL-5 gene promoter functions using transfected IL-5 promoter-reporter constructs, electrophoretic mobility shift assays, and DNase I footprinting.

The in vivo activity of R146225 was first assessed in mice challenged with the 145-2C11 anti-CD3. When i.v. administered to genetically susceptible mice, this antibody induces a strong T-cell activation, evidenced by the appearance in peripheral blood of T-cell-derived cytokines (documented are IL-2, IL-4, IL-6, IL-10, IFN-γ, and tumor necrosis factor-α) and by the splenic accumulation of cytokine mRNA (documented are IL-4 and IL-10) (Flamand et al., 1990; Alegre et al., 1991; Durez et al., 1993; Matthey et al., 1995). Our data show that anti-CD3 treatment of Balb/c mice elicited the systemic release of IL-5 and induced the splenic expression of IL-2, IL-4, IL-5, and IFN-γ mRNA. R146225 inhibited the in vivo IL-5 protein synthesis with high oral potency (lowest effective dose 0.16 mg/kg), but its activity was partial (40–60% inhibition) and evident only at the later times (6 and 8 h) after antibody challenge. The lack of effect at 2 and 4 h may be explained by the anti-CD3-induced release of preformed IL-5 protein and/or translation of present IL-5 mRNA into releasable protein. The presence of IL-5 mRNA, albeit at low abundance, in the spleen of naive mice (Fig. 8, lane 1) may support this explanation. The in vivo effects of R146225 on cytokine synthesis largely resemble its in vitro activity. At the message level, R146225 abrogated the mRNA levels of IL-5, but not those of the other cytokines. At the protein level, the test compound reduced IL-5 production, had little or no effect on the generation of IL-2 and IL-4, but enhanced IFN-γ generation.

According to the Th1/Th2 paradigm (Mosmann and Coffman, 1989), activated helper T (Th) lymphocytes are subdivided into at least two functionally opposing subsets: Th1 cells that secrete IL-2 and IFN-γ, and Th2 cells that produce the “proallergic” IL-4 and IL-5. IFN-γ promotes the development of Th1 cells and simultaneously suppresses Th2 cell-driven allergic inflammation. Indeed, administration of aero- solized IFN-γ to allergen-challenged sensitized mice abrogated pulmonary eosinophilia and normalized airway function (Lack et al., 1996). Thus, R146225’s ability to enhance IFN-γ production, together with its suppression of IL-5 biosynthesis, might synergize to a more efficient attenuation of Th2 responses. A typical Th2-mediated in vivo effect is the accumulation of eosinophils in inflamed tissues and, as such, it is important that R146225 was found to halve the pulmonary eosinophil recruitment into the lungs of C. neoformans-infected mice. In this microbe-initiated disease model, treatment with anti-IL-5 antibody totally prevented eosinophil recruitment, but also attenuated (by 30–60%) the accumulation of lymphocytes and macrophages (Huffnagle et al., 1998). R146225 treatment did not result in such reduction in mononuclear cells, which may be due to its incomplete reduction of lung eosinophilia. Remaining eosinophils may indeed represent a source of chemokines, capable of attracting lymphocytes and macrophages into the lung. Whether proinflammatory functions of these lung leukocytes could be affected by the potential R146225-induced IFN-γ formation is being scrutinized. In summary, the pharmacological activities of R146225 can be characterized as follows: inhibition of IL-5 protein synthesis and IL-5 mRNA expression, enhancement of IFN-γ protein generation, and ability to reduce pulmonary eosinophilia in C. neoformans-exposed mice. On the basis of these data, R146225 may be of potential use in the therapy of eosinophil-driven disorders.

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Send reprint requests to: Jean Van Wauwe, Janssen Research Foundation, Turnhoutseweg 30, 2340 Beerse, Belgium. E-mail: jvwauwe@jansh-jnj.com