Pulmonary Biology of Anti-interleukin 5 Antibodies

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Interleukin 5 (IL-5) is a critical cytokine for the maturation of eosinophil precursors to eosinophils in the bone marrow and those eosinophils then accumulate in the lungs during asthma. We have studied anti IL-5 antibodies on allergic responses in mice, guinea pigs and monkeys and are extending this experiment into humans with a humanized antibody. In a monkey model of pulmonary inflammation and airway hyperreactivity, we found that the TRFK-5 antibody blocked both responses for three months following a single dose of 0.3 mg/kg, i.v. This antibody also blocked lung eosinophilia in mice by inhibiting release from the bone marrow. To facilitate multiple dosing and to reduce immunogenicity in humans, we prepared Sch 55700, a humanized antibody against IL-5. Sch 55700 was also active against lung eosinophilia in allergic monkeys and mice and against pulmonary eosinophilia and airway hyperresponsiveness in guinea pigs. Furthermore, as opposed to steroids, Sch 55700 did not cause immunosuppression in guinea pigs. Studies with this antibody in humans will be critical to establishing the therapeutic potential of IL-5 inhibition.

Key words: eosinophils - interleukin-5 - pulmonary inflammation - antibodies - airway hyperresponsiveness

There is strong but circumstantial evidence for a role of interleukin-5 (IL-5) in asthma, because IL-5 is an essential cytokine for the maturation of eosinophil precursors to eosinophils and it is those eosinophils that accumulate in the lungs during pulmonary inflammation (Gleich et al. 1995). Inhibiting the actions of IL-5 should, therefore, block the maturation of eosinophil precursors to eosinophils and remove at least one of the major causes of asthma. We have investigated the effects of anti-IL-5 antibodies on allergic responses in mice, in guinea pigs and in monkeys and will be extending this experimental paradigm into man using a humanized antibody against IL-5. Using that antibody, we will acquire a good deal of concrete information regarding the role of IL-5 in asthma.

The concept behind using an antibody to inhibit the action of a cytokine is that it binds the ligand making it inaccessible to its receptor. It should, therefore, bind the cytokine with a higher affinity than the receptor itself and, in the case of the antibodies described herein, such is the case. TRFK-5 antibody was raised against purified murine IL-5 (Schumacher et al. 1988) and has an IgG_1 isotype. We have also prepared a CDR grafted humanized antibody against IL-5 named Sch 55700. Both of them have K_D values of about 10^{-10} M and both of them neutralize IL-5 from a variety of species.

In our monkey model of asthma, adult cynomolgus monkeys were tranquilized with ketamine, injected intravenously with the antibody of choice, anesthetized and intubated with an endotracheal tube through which we inserted a pediatric bronchoscope in order to conduct broncheoaveolar lavage with two 10 ml saline washes (Mauser et al. 1995). At the same time, we evaluated the responsiveness of the lungs to a dose response of aerosol histamine. These monkeys were naturally sensitive to aerosol Ascaris antigen, which was used as the allergic stimulus. Twenty-four hour later, bronchoalveolar lavage and airway hyperreactivity were evaluated again such that each monkey served as its own control. In a set of six monkeys, airway hyperreactivity and bronchoalveolar lavage eosinophils were measured in the absence of the antibody. Two weeks later, when the pulmonary parameters in this set of monkeys had returned to baseline, the experiment was repeated in the presence of the antibody, such that this set of monkeys was internally controlled.

To quantitate airway hyperreactivity, we measured the histamine concentration required to cause a 40% decrease in dynamic compliance, the $C_{Dyn}40$, and defined a shift ratio as the $C_{Dyn}40$ before divided by the $C_{Dyn}40$ after Ascaris challenge. A shift greater than 1 indicates hyperreactivity while a shift of 1 indicates that the status of the lungs is the same before and after the allergic challenge.

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Using this experimental protocol, we examined the effects of 0.3 mg/kg of the TRFK-5 antibody on Ascaris induced pulmonary eosinophilia and airway hyperreactivity in monkeys (Table) (Mauser et al. 1995). In the control experiment, we treated the six animals with histamine to establish the pre-Ascaris $C_{Dyn}40$, which turned out to be 0.04% histamine. Subsequent to the allergic challenge, we measured the response to histamine again and established a shift ratio of 7.6 fold. In these monkeys, lavage eosinophil levels were 14 x 10^3/ml before the allergic challenge and 4-5 fold higher after the Ascaris challenge. Two weeks later when these animals had largely returned to normal, the same experiment was performed in the presence of 0.3 mg/kg TRFK-5 antibody. The pre-Ascaris $C_{Dyn}40$ was 0.03 %, virtually the same as previously. However, instead of the shift of 7-8 fold, the shift ratio was 0.74, indicating that the monkey's lungs were not hyperreactive. Baseline eosinophils were about 52,000/ml, but there was only about a 2-fold increase. Irrespective of whether the decrease in lung eosinophilia caused the reduced hyperreactivity, anti-IL-5 treatment inhibited the airway hyperreactivity and this physiology should translate into meaningful results in human asthma.

TABLE

Effect of 0.3 mg/kg TRFK-5 on pulmonary antigen-induced eosinophilia and airway hyperreactivity in monkeys

Treatment	C_{Dyn40} (% histamine)		EOS (10^3 cell/ml)	
	Pre Ascaris	Shift	Pre Ascaris	Increase (%)
Control	0.039±0.009	7.63±3.72	13.8±9.6	460±145
TRFK-5	0.032±0.016	0.74±0.41	52.6±36.6	96±39

In Fig. 1, these data are shown in bar graph format instead of tabular form, where the difference in the eosinophils is the number of bronchoalveolar lavage eosinophils after the allergic challenge minus the number before. The bar on the left represents the untreated animals, where there was a difference of 45,000 eosinophils/ml. As in the Table, when these animals were treated acutely with the TRFK-5 antibody there was about 75% inhibition of the eosinophil infiltration. Despite treating the monkeys with TRFK-5 antibody only once, eosinophilia in these animals was still suppressed as extensively three months later as for the acute treatment. After six months, the eosinophil response largely recovered to normal and the eosinophilia was blocked by a second treatment with the TRFK-5 antibody. This phenomenon was

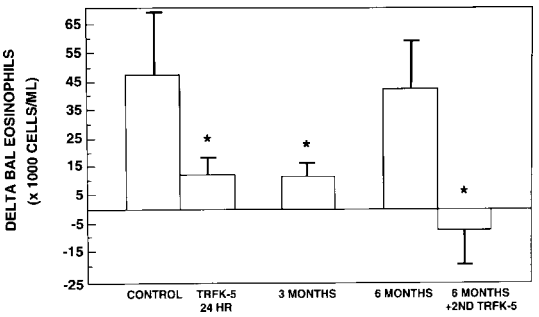

Fig. 1: the effect over six months of a single dose of 0.3 mg/kg TRFK-5 on monkey eosinophilia.

not unique to the eosinophilia, because, after three and six months, the animals had not reestablished their hyperreactivity response to the control level of airway hyperreactivity. However, at six months, they did respond to a second treatment with the TRFK-5 antibody.

In order to determine whether this phenomenon was peculiar to the monkey and to extend the experimental paradigm into a species that is more experimentally tractable, we have studied the extended duration of action of the TRFK-5 antibody in the B6D mouse. Mice were sensitized to alum precipitated ovalbumin by a single 8 μg intraperitoneal injection. Five days later, they were boosted with the same dose to enhance the responsiveness. After 12 days, when IgE levels had increased about 7-fold, they were challenged with aerosol ovalbumin, causing an allergic response. Twenty-four hours after the challenge the mice were sacrificed and specimens of lavage fluid, lung tissue, blood and bone marrow were analyzed for total cells and eosinophils. Antibody was administered 2 hr prior to the ovalbumin challenge.

Using this model, we examined the effect of a single intraperitoneal dose of 1 mg/kg of the TRFK-5 antibody on bronchoalveolar lavage eosinophilia over an extended period of time (Fig. 2) (Egan et al. 1997). Each time point was normalized to the sensitized challenged animals (100%) to avoid age related changes in the response. Throughout the study, the non-sensitized mice had virtually no lavage eosinophils. At the 2 hr time point, the sensitized challenged mice treated with TRFK-5 antibody had their eosinophil response attenuated about 80% while the mice treated with the isotype matched control antibody, GL113, continued to show an eosinophil infiltrate. The same pattern was observed at 2 weeks, 4 weeks, 8 weeks and 12 weeks, but at 24 weeks the mice largely returned to normal with TRFK-5 no longer inhibiting the eosinophil infiltrate. Therefore, as with the mon-

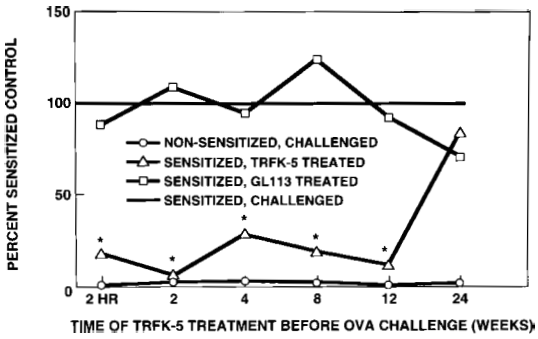

Fig. 2: the effect over six months of a single dose of 1 mg/kg TRFK-5 on BAL eosinophilia in allergic mice.

key, it took several months for the effect of a single dose of the TRFK-5 antibody to dissipate in the mouse.

During this interval, we also examined bone marrow eosinophils in the femur, because the TRFK-5 antibody is known to inhibit release of mature eosinophils into the circulation, which is why they never appeared in the bronchoalveolar lavage fluid (Fig. 3) (Kung et al 1995). Normalized to the sensitized challenged animals, the bone marrow eosinophils in the non-sensitized mice were significantly higher, because the sensitized challenged mice released eosinophils from their bone marrow more rapidly than it could replace them. Starting from a baseline of about 200,000 eosinophils per femur, the sensitized challenged animals dropped to about half that value. The TRFK-5 antibody blocked the change, while the GL-113 antibody had no impact. This effect continued for 2, 4, 8 and possibly 12 weeks, but the mice returned to normal by 24 weeks. Although we have not yet resolved the mechanism for this observation, it certainly will have an impact on the design of clinical trials with the humanized antibody.

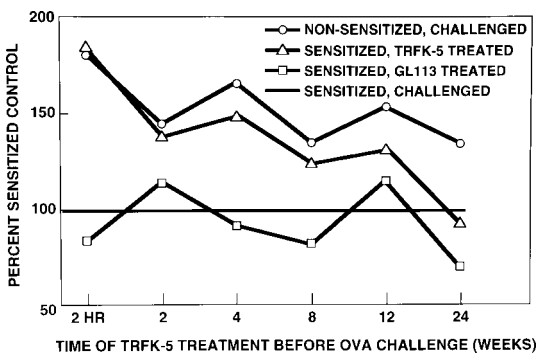

Fig. 3: the effect over six months of a single dose of 1 mg/kg TRFK-5 on bone marrow eosinophilia in allergic mice.

To conduct similar studies in the human, we would need a humanized antibody against IL-5 to facilitate multiple dosing and to reduce immunogenicity. We, therefore, performed CDR grafting based on the 39D10 antibody. The CDR's from 39D10 were grafted onto an otherwise human framework with an IgG_4 isotype and a few framework residues in the region of the CDR's in order to achieve maximum binding affinity. This antibody, named Sch 55700, was first tested for its innate activity to bind human IL-5 compared to 39D10 (Egan et al. 1995). Using a BIAcore binding assay, the on rates for both 39D10 and Sch 55700 were virtually identical at $5 \times 10^5 M^{-1}sec^{-1}$. The off rates between 3 and $4 \times 10^{-5}sec^{-1}$ gave K_D values of 5 and $8 \times 10^{-11}M$, respectively. To evaluate the neutralizing capacity, we tested antibody based inhibition of IL-5 induced proliferation of the TF-1 human erythroleukemic cell line. Again, both antibodies were active in the range of $4 \times 10^{-11}M$. Hence, Sch 55700 is as potent as 39D10, and this binding affinity is stronger than the association between IL-5 and its high affinity receptor, which has a K_D of about $25 \times 10^{-11}M$.

As with TRFK-5, intravenous Sch 55700 was examined in the monkey model of eosinophilia described previously (Fig. 4). In the control period, these monkeys had a pulmonary eosinophilic response of about 225,000 eosinophils/ml in the lavage. Following treatment with 0.3 mg/kg Sch 55700, the acute response was reduced to 40,000 eosinophils/ml, about 80% inhibition of the eosinophil infiltrate. This reduced response continued for six months following a single treatment of Sch 55700, as it did with TRFK-5.

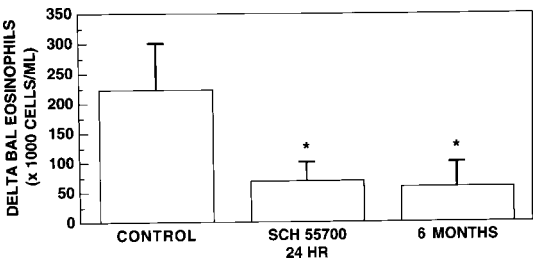

Fig. 4: the effect over six months of a single dose of 0.3 mg/kg Sch 55700 on monkey eosinophilia.

Sch 55700 was also active against lung eosinophlia in allergic mice using the experimental protocol described previously (Fig. 5). When challenged, mice that were not previously sensitized did not mount an eosinophilic response, while those that were both sensitized and challenged gave a massive eosinophil infiltrate that was blocked by 1 mg/kg, i.p. Sch 55700 or 1 mg/kg, i.p. 39D10, but not by lower doses of either antibody.

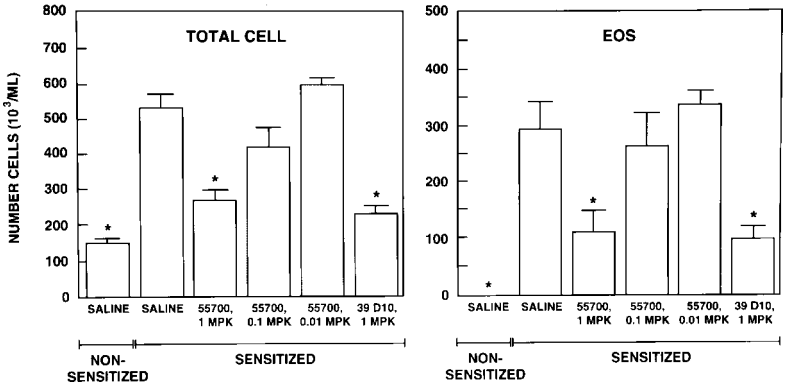

Fig. 5: inhibition by Sch 55700 of the total cells and eosinophils in the BAL of allergic mice.

Sch 55700 was also examined in guinea pigs, because it was possible to study both pulmonary eosinophilia and airway hyperreactivity in that species. The experimental paradigm was very similar to the mouse. The guinea pigs were sensitized to intraperitoneal ovalbumin then challenged with aerosol ovalbumin, following treatment with the antibody or with a sham injection. We then examined lung tissue eosinophilia, lavage eosinophilia and reactivity of the guinea pigs airways to substance P. Fig. 6 shows the effects of intraperitoneal Sch 55700 on both eosinophil infiltration and airway hyperreactivity as indicated by either $C_{Dyn}40$ or specific lung resistance (R_L100). The eosinophil infiltrate was inhibited by 0.03 mg/kg, while it took 1 mg/kg before the physiology was altered. Both the eosinophil infiltration and the airway hyperreactivity were blocked completely at high doses of Sch 55700.

Selective inhibition of IL-5 by an antibody should be less immunosuppressive than steroid treatment and that can be measured by comparing the impact of Sch 55700 compared to steroids on circulating lymphocyte and granulocyte counts in guinea pigs. In guinea pigs, intraperitoneal betamethasone doubled the granulocyte counts and halved the lymphocyte counts over a period of 4 hr following treatment. Betamethasone is a systemically active steroid and such immunosuppression probably does not apply to topical steroids, but it indicates that steroids in general have the potential to cause immunosuppression. On the other hand, Sch 55700 did not alter either granulocyte or lymphocyte numbers for at least three days after intraperitoneal administration at the very high dose of 30 mg/kg (Fig. 7). Therefore, as opposed to steroids, Sch 55700 does not even have the potential to cause the immunosuppression seen with steroids in the guinea pig.

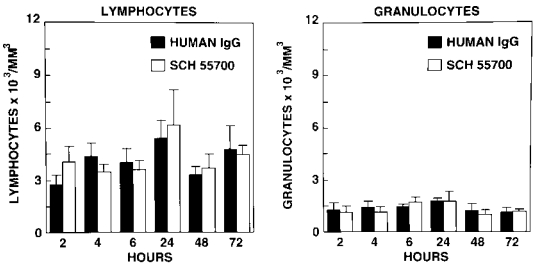

Fig. 7: the effect of Sch 55700 on circulating lymphocytes and granulocytes.

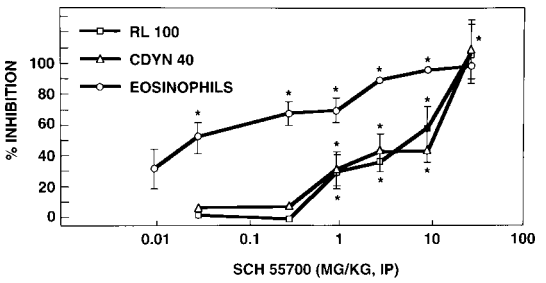

Fig. 6: inhibition by Sch 55700 of the bronchial hyperresponsiveness and the pulmonary eosinophilia in allergic guinea pigs.

In summary, we have measured eosinophilia and airway hyperreactivity in monkeys, mice and guinea pigs in response to an allergic challenge. Antibodies to IL-5 can completely inhibit the eosinophilia and the airway hyperreactivity. When we started these studies, it was thought that GM-CSF or IL-3 could substitute in eosinophilopoiesis if IL-5 was inhibited, but certainly that is not the case. The duration of action of the antibodies is between three and six months, and we are working on the mechanism of extended duration. Nevertheless,

this simple empirical observation will certainly help dictate our clinical protocol. Anti-IL-5 antibodies inhibit release of eosinophils from the bone marrow and that mechanism holds for the 3-6 months of biological activity. Lastly, Sch 55700 is a CDR grafted humanized antibody against IL-5 that will proceed for testing against clinical asthma.

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