



Cloning, expression and biological activity of equine interleukin (IL)-5

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Abstract

The cytokine, interleukin (IL)-5 stimulates eosinophil differentiation, activation and survival and can prime these cells, increasing the response to other mediators. In view of its many effects on eosinophils, IL-5 has been implicated in the pathogenesis of allergic disease in man. Here we report the cloning of equine IL-5 and expression of the recombinant protein by transfection of Chinese hamster ovary (CHO) cells. The cloned cDNA sequence consisted of 405 nucleotides and encoded a protein of 135 amino acids. There is >85% identity with feline, bovine, ovine, canine, and human IL-5 sequences at the nucleotide and protein level. Supernatants containing equine IL-5 were also examined for biological activity. CHO supernatant containing equine recombinant (eqr) IL-5, like the human ortholog (hrIL-5), induced concentration dependent equine eosinophil adherence to autologous serum-coated plastic ($9.7 \pm 1.5\%$ with a 1:100 dilution of eqrIL-5 and $9.1 \pm 1.6\%$ adherence with 1 nM hrIL-5; $n = 4$). The eqr protein also caused concentration dependent superoxide production (11.9 ± 2.4 nmol {reduced cytochrome (cyt) C}/ 10^6 cells at a 1:50 dilution, $n = 4$). In contrast, hrIL-5 only caused significant superoxide production when diluted in conditioned CHO medium, an effect that was inhibited by the anti-human mAb, TRFK5 (4.4 ± 0.3 versus 0.3 ± 0.4 nmol/ 10^6 cells for 0.5 nM hrIL-5 in the presence of the isotype matched IgG₁ control (10 μ M) and TRFK5 (10 μ M), respectively). TRFK5 also significantly inhibited hrIL-5 induced adherence at concentrations of 0.3 μ g/ml and above but had no significant inhibitory effect on either superoxide or adherence caused by eqrIL-5. These results demonstrate that equine IL-5 expressed by CHO cells stimulates equine eosinophils, suggesting that this cytokine could play a role in eosinophil recruitment and activation in equine allergic disease. The anti-human and murine moAb TRFK5 does not appear to recognise the equine protein.

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Keywords: Interleukin (IL)-5; Eosinophil; Adherence; Superoxide; Horse

Abbreviations: ANOVA, analysis of variance; CHO, Chinese hamster ovary; cyt C, cytochrome C; HBSS, Hanks Balanced Salt Solution; H, histamine; mRNA, messenger ribonucleic acid

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1. Introduction

Eosinophils are known to play a key role in host defence against parasite infestation but are also

considered to be important effector cells in the pathogenesis of human allergic disease (Gleich, 2000). Eosinophils accumulate in the skin of allergic horses with insect hypersensitivity following exposure to *Culicoides* antigen and have been implicated in the pathogenesis of this condition (Riek, 1953; Kurotaki et al., 1994; Foster et al., 1995; Foster and Cunningham, 1998a; McKelvie et al., 1999). Circulating eosinophil numbers have also been reported to be increased in horses with clinical signs of insect hypersensitivity although this is not a consistent finding (Riek, 1953; Foster et al., 1995). In contrast to equine allergic skin disease and human allergic asthma, eosinophils are rarely seen in the lungs of horses with recurrent airway obstruction (RAO), an hypersensitivity to inhaled antigens (Robinson et al., 1996).

Little is known at present concerning the mediators responsible for the recruitment of equine eosinophils to, and their activation in, the tissues. Increased messenger ribonucleic acid (mRNA) expression for eotaxin, a potent eosinophil chemoattractant, has been reported in the intestinal tissue of healthy animals where large numbers of eosinophils were detected (Benarafa et al., 2000). There is also evidence of increased eotaxin mRNA expression in the lesional skin of ponies with insect hypersensitivity when eosinophils are present in the lesions (Benarafa et al., 2002). Histamine (H) may also contribute to antigen-induced eosinophil recruitment in this condition as the H₁ receptor antagonist, chlorpheniramine, caused a reduction in *Culicoides* antigen-induced eosinophil accumulation in the skin of affected ponies (Foster et al., 1998). However, this effect may be due to inhibition of vascular H₁ receptors as intradermal histamine caused little eosinophil accumulation, and only at high concentrations. Moreover, chlorpheniramine also reduced *Culicoides* antigen-induced neutrophil accumulation and there is no evidence that histamine causes adherence or migration of these cells (Foster and Cunningham, 1998b; Foster et al., 1998).

Interleukin (IL)-5 exerts a variety of effects on eosinophils which range from inducing differentiation in the bone marrow to enhancing cell survival time; adherence, migration, degranulation, and superoxide production have all been reported following exposure of mature eosinophils to IL-5 (Weltman and Karim, 2000). IL-5 also causes eosinophil priming, enhancing

the effects of other mediators. This is thought to be a feature of human allergic disease as blood eosinophils from allergic subjects behave similarly to eosinophils from normal subjects that have been primed in vitro with cytokines such as IL-5 (Koenderman et al., 1996). IL-5 is produced by activated Th2 lymphocytes and Giguere and Prescott (1999) have shown that mitogen activated equine blood mononuclear cells express mRNA for a range of cytokines, including IL-5. Interestingly, despite the lack of evidence for eosinophil recruitment to the lungs of horses with clinical signs of RAO, Lavoie et al. (2001) have recently reported increased expression of mRNA for IL-5 by bronchoalveolar lavage cells from these animals when compared to control horses and these authors have suggested that RAO is characterised by a Th2-type cytokine profile. By contrast, horses affected with summer pasture associated-obstructive pulmonary disease (SPAOPD), another form of equine RAO, do not express IL-5 mRNA either in bronchoalveolar or peripheral blood mononuclear cells (Beadle et al., 2002). As detection of IL-5 protein is not possible at present, the role of this cytokine in equine allergic airway disease remains uncertain. T-lymphocytes have also been implicated in the pathogenesis of equine insect hypersensitivity (Kurotaki et al., 1994; McKelvie et al., 1999) although the subsets have yet to be identified. Eosinophils, may also be a source of IL-5, as demonstrated by Tanaka et al. (1994), who showed up-regulation of IL-5 mRNA and immunoreactive protein expression by activated eosinophils in the lesional skin of human patients with atopic dermatitis. These cells accumulate in large numbers in developing lesions of horses with insect hypersensitivity. Whether equine eosinophils can release IL-5 remains to be determined although these cells appear to be capable of producing cytokines as TNF α - and IL-1 β -like biological activity were measured in the supernatant of activated cells (Bailey and Cunningham, 2001a).

Human recombinant (hr) IL-5 has been shown to induce equine eosinophil adherence to protein coated plastic (Foster et al., 1997). Moreover the hr protein increased the adherence of these cells to cultured equine vascular endothelial cells by acting directly on the eosinophil (Bailey and Cunningham, 2001a,b). Interestingly, IL-5 induced adherence of eosinophils from ponies with insect hypersensitivity to endothelial

cells that had been pre-treated with IL-1 β was significantly greater than that of cells from normal ponies during the active phase of the disease when clinical signs were present. This could suggest a role for IL-5 in the disease process. In order to investigate the role of IL-5 further it is desirable to utilise the equine protein as binding affinities of other species homologues may vary (Scott et al., 2000), as may the efficiency of intracellular signal transduction mechanisms. This study reports the cloning of equine IL-5 and expression of the recombinant cytokine in Chinese hamster ovary (CHO) cells. The supernatant containing equine recombinant (eqr) IL-5 has been examined for biological activity using assays of eosinophil adherence and superoxide production.

2. Materials and methods

2.1. Reagents

Percoll was obtained from Amersham Pharmacia Biotech Ltd., Bucks, UK. The anti-human/murine mAb, TRFK5 (no azide; low endotoxin), which also neutralises ovine IL-5 (Stevenson et al., 1998), and a rat IgG₁ isotype matched control were obtained from Becton Dickenson (Oxford, UK). Unless otherwise stated, all other media and chemicals were purchased from Sigma Chemical Company (Dorset, UK or St. Louis, MO) or BDH Ltd., Poole, UK. The DHFR⁻ CHO cells were obtained from the American Type Culture Collection, Manassas, VA.

2.2. Cloning

The cDNA encoding equine IL-5 was amplified from pokeweed mitogen (PWM)-stimulated equine peripheral blood mononuclear cells (PBMCs). The 5'-(atgaggatgcttctgcatttga) and 3'-(tcagccttctattgtc-cactc) primers were deduced from other species' IL-5 sequences reported in GeneBank. The amplified product was cloned into the pCR2.1 plasmid using the TA Cloning Kit (Invitrogen, Carlsbad, CA). The resulting plasmid, pCREQIL5, was sequenced by chain-termination sequencing according to the Sequenase Version 2.0 kit (United States Biochemical, Cleveland, OH). Two additional clones prepared from different cDNA preparations were also sequenced and

found to be identical. The nucleotide sequence was compared to the other known IL-5 sequences using BLAST (Altschul et al., 1990).

2.3. Expression

Equine IL-5 cDNA was excised from pCREQIL5 and the gel purified fragment ligated into the eukaryotic expression vector pED (a kind gift from Dr. R. Kaufman, Genetics Institute, Cambridge, MA) such that translation was initiated at the first start codon within the cloned equine cDNA fragment. This vector contains the dihydrofolate reductase (DHFR) gene which, when transfected into DHFR⁻ CHO cells (ATCC# CRL 9096), allows for growth under the selective pressure of aminopterin (Kaufman and Sharp, 1982). The DHFR⁻ CHO cells were propagated in α MEM containing 10% (v/v) heat inactivated, dialysed foetal bovine serum and supplemented with adenosine, deoxyadenosine and thymidine. The cells were transfected with pEDEQ5 using calcium phosphate-based transfection and glycerol shock. The transfected cells were selected by growth in unsupplemented α MEM containing dialysed serum at 37 °C with 5% CO₂. The transfected CHO cells were cloned by limiting dilution in 96-well flat bottom plates. Several clones expressing equine IL-5 mRNA were picked for continued propagation and selection with aminopterin. Supernates were continuously collected from these expanded clones and stored frozen until assayed for biological activity.

3. Biological activity

3.1. Eosinophil separation

Eosinophils were separated by Percoll density gradient centrifugation from healthy New Forest ponies, as previously described (Foster and Cunningham, 1997). Earlier studies have shown that cells separated by this technique are >99% pure and differential counts on some, but not all, of the preparations used in these studies confirmed that the purity was at least 98%. Cells were suspended at a final concentration of 10⁶ ml⁻¹ in Hanks Balanced Salt Solution (HBSS) containing 10 mM HEPES buffer, 0.125% BSA and 40 mM sodium bicarbonate (HBSS complete) for

adherence assays and in HBSS complete containing 2.5 mg/ml cytochrome (cyt) C for superoxide assays.

3.2. Adherence assay

Adherence of equine eosinophils to autologous serum-coated plastic was measured using a colorimetric assay for eosinophil peroxidase (EPO), as described previously (Foster et al., 1997). The adherence-inducing activity present in CHO supernatant containing eqrIL-5 that was diluted in PBS:0.25% BSA (1:1000–1:50) was measured after a 30 min incubation. hrIL-5 (0.3 pM–30 nM) diluted in PBS:0.25% BSA was used as the positive control. In order to determine whether the effects of eqrIL-5 could be neutralised by TRFK5, the moAb (0.01–10 µg/ml) was added to eqrIL-5 (1:100 dilution) or hrIL-5 (0.3 nM), placed in the wells and warmed at 37 °C for 10 min before the eosinophils were added. An isotype matched IgG₁ antibody (1 and 10 µg/ml) was used as the control. Adherence has been expressed as a percentage of the total number of cells (10⁵) added to each well.

3.3. Superoxide assay

Equine eosinophil superoxide production was measured by the reduction of cyt C using a previously described colorimetric assay (Foster and Cunningham, 1997). Cells were incubated with eqrIL-5 for 30 min at 37 °C and histamine (10⁻⁴ M) was used as the positive control. In the first experiment, the effect of CHO

supernatant containing eqrIL-5, diluted in PBS:0.25% BSA (1:1000–1:10) was examined. In a second experiment, conditioned CHO medium from mock-transfected CHO cells was used as the control and 2-fold dilutions of CHO supernatant containing eqrIL-5 examined over the range 1:400–1:10. In a third study, the effects of TRFK5 (0.01–10 µg/ml) or the isotype matched control antibody (10 µg/ml) on the response to a 1:50 dilution of CHO supernatant containing eqrIL-5 was tested.

In a separate study, for comparison, the effect of hrIL-5 (0.01–1 nM) on superoxide production by equine eosinophils was examined. As the hr protein had no significant effect on superoxide production but eqrIL-5 did produce a response (Fig. 3), the effect of diluting the hr protein (0.01–1 nM) in conditioned CHO medium (1:50 dilution) on superoxide production was examined. The effect of TRFK5 (0.1–10 µg/ml) on the response to hrIL-5 (0.5 nM) in conditioned CHO medium (1:50 dilution) was also tested. Superoxide production has been expressed as nmol {of reduced cyt C}/10⁶ cells.

3.4. Effect of heating on the biological activity of IL-5

The effects of heating CHO supernatant containing eqrIL-5 (1:50 dilution) to 56 and 95 °C on the superoxide-inducing activity and heating to 95 °C on the adherence-inducing activity were also examined. As only a partial reduction in activity was observed in each assay (Table 1), the effects of heating hrIL-5

Table 1

The effect of heating on superoxide- and adherence-inducing activity of eqrIL-5 and hrIL-5^a

	Superoxide production (nmol/10 ⁶ cells)				Adherence (%)		
	<i>n</i>	25 °C	56 °C	95 °C	<i>n</i>	25 °C	95 °C
eqrIL-5 (1:50)	3	8.9 ± 2.1	9.5 ± 2.9	4.0 ± 1.1	5	9.3 ± 1.3	7.3 ± 1.2
CHO (1:50)	3	0.4 ± 1.9		0.3 ± 0.2	5	1.7 ± 0.5	1.4 ± 0.5
1 nM hrIL-5 in CHO (1:50)					4	13.4 ± 2.4	6.1 ± 3.2
CHO (1:50)					4	2.3 ± 0.4	1.2 ± 0.2
1 nM hrIL-5 in PBS:0.25% BSA					4	9.9 ± 2.4	1.3 ± 0.3**
PBS:0.25% BSA					4	1.5 ± 0.5	ND ^b

^a eqrIL-5 in CHO supernatant was incubated at different temperatures for 30 min before measurement of equine eosinophil superoxide production or adherence. hrIL-5 in PBS:0.25% BSA or a 1:50 dilution of CHO conditioned medium was incubated for 30 min at 25 or 95 °C prior to measuring equine eosinophil adherence. Values are the means obtained using eosinophils from 3 to 5 different ponies ± SEM.

^b Not determined.

** *P* < 0.001 when compared to activity at room temperature; Student's *t*-test.

(1 nM) in PBS:0.25% BSA and hrIL-5 in CHO conditioned medium (1:50 dilution) to 95 °C were also examined in the adherence assay.

3.5. Statistical analysis

The significance of the effects of eqr and hrIL-5 on adherence and superoxide production and the effects of TRFK5 were examined using repeated measures analysis of variance (ANOVA) followed by Dunnett's post hoc test. The effect of heating on the biological activity of eqrIL-5 and hrIL-5 was examined using Student's *t*-test. Significance was accepted when the *P*-value was less than or equal to 0.05.

4. Results

4.1. Cloning and expression of equine IL-5

The cloned cDNA sequence for equine IL-5 consists of 405 nucleotides encoding a 135 amino acid protein. The complete sequence for equine IL-5 is available on GeneBank (U91947). There is >85% identity with feline, bovine, ovine, canine, and human IL-5 sequences at the nucleotide and protein level. In order to confirm biological activity, the corresponding cDNA sequence was subcloned into the pED expression vector and expressed in CHO cells.

4.2. IL-5 induced adherence

As has been previously demonstrated (Foster et al., 1997), hrIL-5 caused concentration dependent adherence of equine eosinophils to serum-coated plastic. CHO supernatant containing eqrIL-5 caused dilution related adherence which was of a similar magnitude to that observed with the purified hr protein (Fig. 1). TRFK5 had no significant effect on adherence caused by eqrIL-5 whereas the activity of hrIL-5 was significantly reduced at antibody concentrations of 0.3 µg/ml and above (Fig. 2).

4.3. IL-5 induced superoxide production

CHO supernatant containing eqrIL-5 also caused concentration dependent superoxide production by equine eosinophils (Fig. 3a). The maximum amount

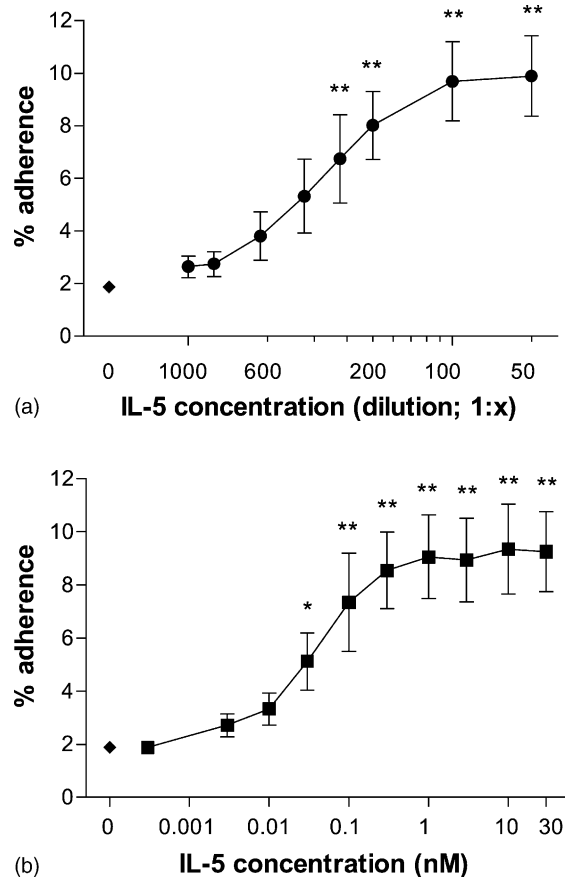


Fig. 1. The effect of (a) eqrIL-5 in CHO supernatant and (b) hrIL-5 on equine eosinophil adherence to autologous serum-coated plastic. Each point is the mean of values obtained using eosinophils from four different ponies and bars represent SEM. Filled diamonds represent the response of unstimulated cells in HBSS. **P* < 0.05; ***P* < 0.01 when compared to the response of unstimulated eosinophils; repeat measures ANOVA followed by Dunnett's multiple comparison test.

of superoxide produced was similar to that seen in response to the positive control, 10^{-4} M histamine (16.0 ± 2.0 nmol cyt C/ 10^6 cells, $n = 4$), a concentration previously shown to produce a maximal response in this assay (Foster and Cunningham, 1997). Concentration dependent superoxide production was also seen when eqrIL-5 was diluted in CHO conditioned medium. At a dilution of 1:10, the highest concentration of CHO supernatant containing eqrIL-5 used in the assay, CHO conditioned medium alone had no effect on superoxide production (Fig. 3b). Superoxide production by equine eosinophils in the presence of

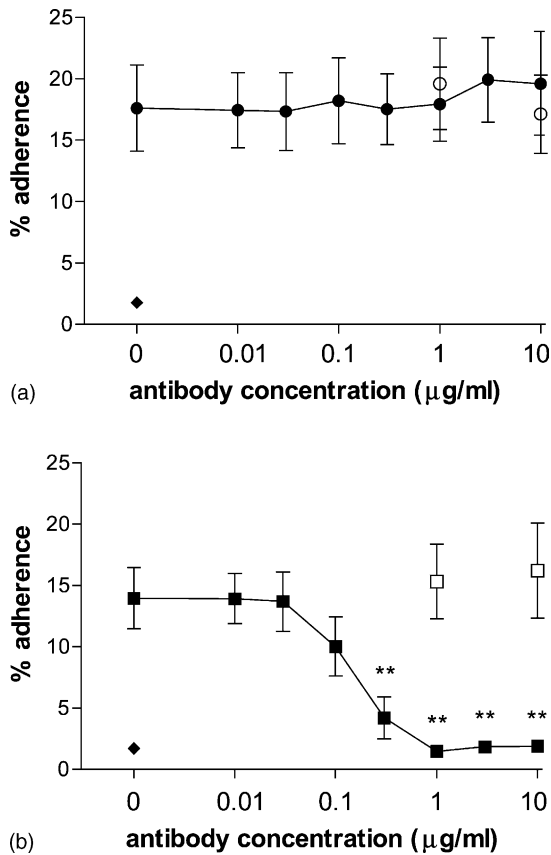


Fig. 2. The effect of TRFK5 mAb on equine eosinophil adherence in response to (a) eqrIL-5 in CHO supernatant (1:100 dilution) and (b) hrIL-5 (0.3 nM). Open circles (a) or squares (b) represent the response in the presence of the IgG₁ isotype control. Each point is the mean of values obtained using eosinophils from four different ponies and bars represent SEM. ** $P < 0.01$ when compared to the response of unstimulated eosinophils; repeat measures ANOVA followed by Dunnett's multiple comparison test.

hrIL-5 diluted in PBS:0.25% BSA was not different to that of unstimulated cells when tested over the concentration range 0.01–1 nM. However, when diluted in conditioned CHO medium (1:50), the hr protein caused significant superoxide production (Fig. 3c). This activity was inhibited by TRFK5 (Fig. 4a) whereas the superoxide-inducing activity in CHO supernatant containing eqrIL-5 was not (Fig. 4b). The lack of inhibition of the biological activity of eqrIL-5 by TRFK5 in the adherence and superoxide assays was supported by negative findings for Western blots and ELISA using the same antibody (data not shown).

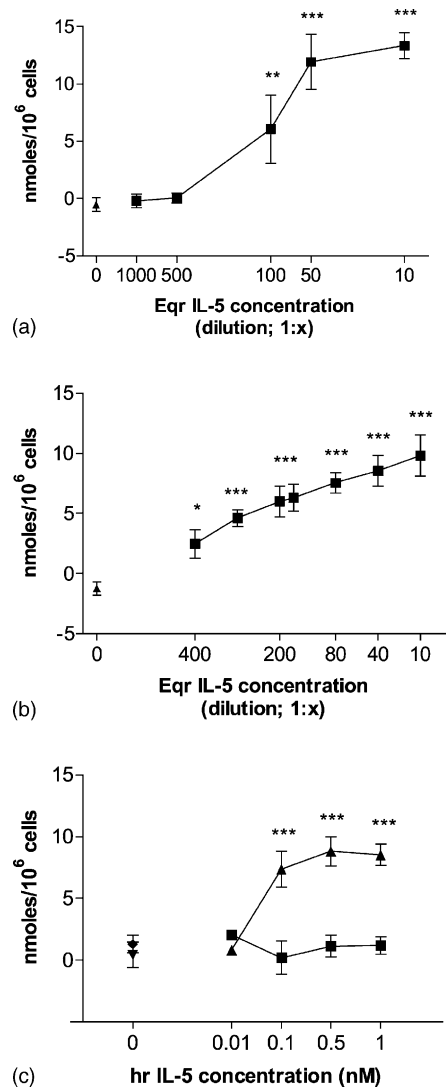


Fig. 3. Superoxide production by equine eosinophils stimulated with (a) CHO supernatant containing eqrIL-5 and diluted in PBS:0.25% BSA, (b) CHO supernatant containing eqrIL-5 and diluted in conditioned CHO medium, and (c) hrIL-5 diluted in PBS:0.25% BSA (squares) or conditioned CHO medium (1:50; triangles). Each point is the mean of values obtained using eosinophils from four (a) and (c) or three (b) different ponies and bars represent SEM. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ when compared to unstimulated eosinophils; repeat measures ANOVA followed by Dunnett's multiple comparison test.

4.4. Effect of heating on the biological activity of IL-5

The amount of superoxide produced by eosinophils stimulated with eqrIL-5 that had been heated to 95 °C

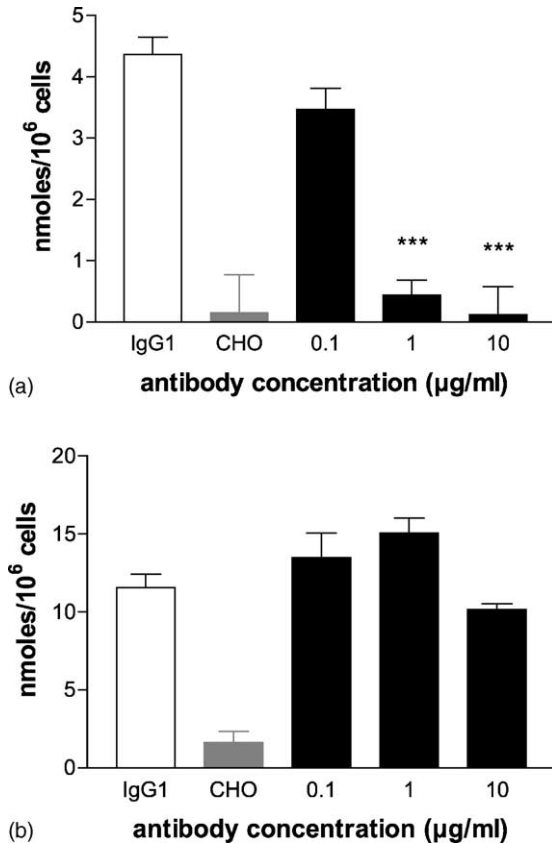


Fig. 4. The effect of TRFK5 on equine eosinophil superoxide production in response to (a) hrIL-5 (0.5 nM) in conditioned CHO medium (1:50 dilution) and (b) CHO supernatant containing eqrIL-5 (1:50 dilution) following treatment with TRFK5 or the isotype matched IgG₁ control moAb. Responses to conditioned CHO medium alone are also shown. Bars represent the mean of values obtained using eosinophils from three different ponies and lines represent SEM. *** $P < 0.001$ when compared to responses in the presence of the control moAb; repeat measures ANOVA followed by Dunnett's multiple comparison test.

for 30 min was reduced by $64 \pm 11\%$ when compared to that seen in response to stimulation with eqrIL-5 that had been incubated for 30 min at 25°C although the reduction was not statistically significant ($P = 0.102$). Heating eqrIL-5 to 56°C did not reduce the amount of superoxide produced (Table 1). Heating to 95°C also reduced eqrIL-5 induced eosinophil adherence but only by $19.4 \pm 8.0\%$. Interestingly, whilst the amount of eosinophil adherence caused by hrIL-5 diluted in PBS:0.25% BSA was reduced by $83.1 \pm 6.1\%$ when the hr protein was heated to 95°C ,

a smaller reduction in adherence ($55.5 \pm 15.5\%$) was caused by heating when the hrIL-5 was diluted in CHO supernatant (Table 1).

5. Discussion

In the first part of this study, equine IL-5 was PCR cloned using consensus sequence primers as previously described for other equine cytokines (Vandergriff and Horohov, 1993; Vandergriff et al., 1994; Swiderski et al., 2000). The cDNA and amino acid sequences of equine IL-5 are highly conserved when compared to those of other species. This includes the conservation of a number of critical epitopes including those recognised by the TRFK5 antibody (see below). Thus it is also likely that for biological function equine IL-5 must also form a homodimer arranged in a head-to-tail configuration, covalently linked by two disulphide bonds via cysteines (McKenzie et al., 1991). While equine IL-5 contains several possible *N*-glycosylation sites, these glycosylation sites are not conserved among species (Kodama et al., 1992) and are not thought to be essential for the biological activity of the protein (Tominaga et al., 1990). The biological activity of this cloned protein was confirmed using assays of equine eosinophil function.

hrIL-5 has previously been shown to cause concentration dependent adherence of equine eosinophils to protein coated plastic (Foster et al., 1997). The results in the present study confirm that hrIL-5 can act directly on equine cells to increase their adhesive properties. The equine ortholog of IL-5 also appears able to stimulate equine eosinophils to adhere to protein coated plastic as CHO supernatant containing eqrIL-5 caused concentration dependent adherence which was of similar magnitude to that seen in response to hrIL-5 over the concentration range tested (Fig. 1). Murine, human and guinea pig IL-5 have been shown to bind with similar affinity (less than 5-fold difference) to the IL-5 receptor on guinea pig eosinophils (Scott et al., 2000). This suggests that the amount of eqrIL-5 present in CHO supernatant could be calculated by extrapolation from the concentration response curve to the hr cytokine. However, despite the similarity between affinities of the different species orthologs for the guinea pig receptor, the affinity of human and guinea pig IL-5 for the

murine receptor was at least a 1000-fold lower (Scott et al., 2000). A comparison of the relative potencies of eqrIL-5 and hrIL-5 must therefore await protein purification.

CHO supernatant containing eqrIL-5 also caused concentration dependent superoxide production. However, neither superoxide production, nor adherence, in response to the eqr protein was neutralised by TRFK5. The antibody cross-reacts with human, murine and ovine IL-5 and has been shown previously to inhibit hrIL-5 induced adherence of equine eosinophils to protein coated plastic, a finding confirmed in the present study (Foster et al., 1997; Stevenson et al., 1998). The lack of inhibition of adherence and superoxide-inducing activity caused by CHO supernatant containing eqrIL-5 could be explained if the mAb was directed against an epitope that is not present on equine IL-5. This is supported by the negative findings on Western blots and ELISA. However, the presumed TRFK5 epitope (Arg92 and Gln95 on human and Arg90 and Gln93 on mouse IL-5) (Dickason et al., 1996) is present in eqrIL-5 (Arg92 and Gln95 being conserved). A similar absence of TRFK5 neutralisation was reported for recombinant porcine IL-5 by Sylvén et al. (2000) and porcine IL-5 also contains the putative monoclonal antibody epitope.

It is possible that the eosinophil activating properties of CHO supernatant were not due to the cytokine. That heating to 95 °C only partially reduced superoxide production induced by eqrIL-5 and had little effect on the adherence-inducing activity suggests that the biological activity observed might additionally involve the action of a component(s) of the conditioned CHO medium. The adherence induced by purified hrIL-5 was almost completely inhibited by heating to 95 °C but, interestingly, the amount of inhibition was smaller when the hrIL-5 was suspended in CHO conditioned medium. It is therefore possible that the conditioned medium protected the recombinant IL-5 protein from the effects of heating.

In contrast to the findings in the adherence assay, hrIL-5 alone did not directly cause significant superoxide production by equine eosinophils. hrIL-5 has been reported to cause superoxide production by human eosinophils (Horie et al., 1996; Ezeamuzie and Al-Hage, 1998) and when diluted in conditioned CHO medium, which alone caused no superoxide production, the hr protein produced a significant

response in equine cells. This response could be inhibited by TRFK5 in a concentration dependent manner suggests that the observed effect was IL-5 dependent. One explanation could be that priming of eosinophils by material present in conditioned CHO medium occurred, increasing superoxide production by hrIL-5. If the affinity of hrIL-5 for the equine receptor was low, it may be that, even at the highest concentration tested, only small amounts of superoxide would be produced. However, hrIL-5 induced equine eosinophil adherence at similar concentrations to those required to stimulate human eosinophils to adhere to protein coated plastic (Ezeamuzie and Al-Hage, 1998). Alternatively, the hr protein may have a similar affinity for the equine receptor as eqrIL-5 but the intracellular signalling events occurring downstream of the receptor which result in superoxide production, may not be activated as effectively by hrIL-5 as those required for adherence so that priming is required for the human cytokine to be effective. Although the mechanism of IL-5 signal transduction has been extensively studied (Adachi and Alam, 1998), post-receptor signalling events resulting in these biological responses have not been determined in equine eosinophils. Interestingly, hrIL-5 caused a higher percentage of eosinophils to adhere when diluted in CHO conditioned medium rather than PBS:0.25% BSA (Table 1). Although the increase was not significant, it supports the suggestion that the effects of eqrIL-5 might be enhanced due to an effect of a component(s) in the medium on equine eosinophils.

Following the addition of the mRNA sequence for equine IL-5 to the GeneBank data base in 1997, several investigators have used this information to enhance our knowledge of equine leucocyte biology and disease pathogenesis (Giguere and Prescott, 1999; Swiderski et al., 1999; Lavoie et al., 2001). Here we provide additional information on the cloning and expression of eqrIL-5, together with evidence that the cytokine activates equine eosinophils.

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