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# Production and characterisation of monoclonal antibodies specific for chicken interleukin-2

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**Abstract**

Using genetic immunisation of mice, we produced antibodies against chicken interleukin-2 (ChIL-2), the first produced against a non-mammalian interleukin. After a final injection with a recombinant ChIL-2 protein, two stable hybridoma cell lines were established which secreted monoclonal antibodies (MAbs) against this cytokine. Specific binding of the two MAbs to recombinant ChIL-2 produced by *Escherichia coli* and COS-7 cells was demonstrated in an indirect ELISA, Western blotting and dot blots. Both of them were able to neutralise the biological activity of the ChIL-2, but neither allowed the detection of ChIL-2 by flow cytometry. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Interleukin-2; Chicken; Monoclonal antibodies; Species-specificity; Neutralising

## 1. Introduction

The formal existence of a T1–T2 paradigm, first postulated for the mouse by Mosmann and Coffman (1989), has yet to be demonstrated in a non-mammalian species. In mammals, type 1 (T1) cells are characterised by the production of interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour necrosis factor-β (TNF-β), which induce macrophage activation, DTH reactions and synthesis of IgG2a (IFN-γ). Type 2 (T2) cells, on the other hand, are characterised by the production of IL-4 and IL-5, stimulating eosinophilia and secretion of IgG1 and IgE. On the basis of the pattern of cytokines produced, T1 and T2 responses have also been described in cattle (Brown et al., 1993, 1994a,b), sheep (Gill et al., 2000) and cats (Pedersen et al., 1998; Sukura et al., 1998).

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In the chicken, there is as yet no evidence for the existence of a T1–T2 paradigm. Chickens lack direct equivalents of eosinophils, there is no evidence yet for IgE, and their IgG equivalent, IgY, does not have different subclasses. Further, despite several good chicken EST libraries now being available, none of the canonical T2 cytokines (IL-4, IL-5 or IL-10) have yet been cloned. Several T1 cytokines have been cloned, however, namely IL-2 (Sundick and Gill-Dixon, 1997; Kaiser and Mariani, 1999), IFN-$\gamma$ (Digby and Lowenthal, 1995) and IL-18 (Schneider et al., 2000). The only anti-chicken cytokine monoclonal antibodies (MAbs) that have been produced so far recognise ChIFN-$\gamma$ (Lambrecht et al., 2000; Yun et al., 2000), and allowed the development of a capture ELISA (Lambrecht et al., 2000).

In the present work, we describe the production of MAbs against ChIL-2 in mice by DNA immunisation, using a method based on that of Weynants et al. (1998).

## 2. Materials and methods

### 2.1. Plasmid expression vectors

A *Not*I fragment containing the ChIL-2 cDNA (Sundick and Gill-Dixon, 1997) was inserted into the *Not*I site in the polylinker of the eukaryotic expression vector pCI-neo (Promega, Southampton, UK) giving rise to pCI-neo-ChIL-2. A similar plasmid, pCI-neo-TuIL-2, containing the turkey (Tu) IL-2 cDNA (Lawson et al., 2000) was generated in the same manner.

A *Bam*HI-*Kpn*I ChIL-2 cDNA fragment was inserted into the *Bam*HI-*Kpn*I site in the polylinker of the prokaryotic expression vector pQE30 (Qiagen, Crawley, UK) giving rise to pQE30-ChIL-2.

### 2.2. Production of recombinant ChIL-2 and TuIL-2 by COS-7 cells

COS-7 cells were routinely grown in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, Paisley, UK) containing 10% FBS (PAA Laboratories GmbH, Linz, Austria), 1% non-essential aa, 1% L-glutamine, 1 U/ml penicillin and 1 μg/ml streptomycin (P/S) at 37 °C, 5% $CO_2$, and passaged using standard conditions (Tregaskes and Young, 1997). Cells were cultured at $5 \times 10^5$/ml for 18–24 h at 37 °C, 5% $CO_2$, and washed twice with phosphate buffered saline (PBS). Five millilitres of serum-free media, containing 7.5 μg/ml DNA (either pCI-neo, pCI-neo-ChIL-2, pCI-neo-TuIL-2 or no plasmid), 258 μg/ml chloroquine and 600 μg/ml DEAE-dextran was then added to the cells. Flasks were incubated for 3 h at 37 °C, 5% $CO_2$. The transfection media was then removed and cells washed once with PBS. PBS containing 10% dimethylsulphoxide was then added for 2 min, removed and replaced with 5 ml growth medium. After 24 h growth at 37 °C, 5% $CO_2$, growth media was replaced with serum-free DMEM. The cells were then incubated for 72 h, following which supernatant was collected and stored at 4 °C prior to use.

### 2.3. Production of recombinant ChIL-2 by E. coli

Cultures (50 ml) were induced with 1 mM IPTG for 4.5 h at room temperature (RT), centrifuged (5000*g*, 15 min) and cell pellets resuspended in 5 ml lysis buffer composed of

50 mM $NaH_2PO_4$, pH 8.0, 300 mM NaCl, 10 mM imidazole. The cell suspension was then frozen in dry ice-ethanol and thawed in cold water. Cells were then digested with 1 mg/ml lysozyme for 35 min on ice. Cells were then sonicated on ice, with a microtip probe, for six 10 s pulses, with intervening 10 s pauses, followed by digestion with 10 μg/ml RNase A and 15 U/ml DNase I (15 min on ice). The lysate was centrifuged (10 000*g*, 30 min at 4 °C) and the supernatant collected contained the soluble fraction, whilst the pellet contained insoluble protein aggregates. The insoluble fraction was then extracted twice (by resuspension and centrifugation), in 1 ml 0.25% Tween-20, 0.1 mM EGTA, and the resulting supernatants combined with the previous supernatant containing the soluble fraction. Analysis of protein solubility by SDS-PAGE and Western blot (with an anti-6 × His MAb) showed that the 6 × His-ChIL-2 fusion protein was present.

Soluble His-tagged fusion protein was purified by affinity chromatography, using a nickel-nitrilotriacetic acid (Ni-NTA) matrix. Purification was under native conditions, following manufacturer's instructions. Essentially, the combined soluble fraction was incubated with 1 ml of a 50% slurry of Ni-NTA coupled to Sepharose CL-6B for 1 h at 4 °C, with continual rol-ling. The slurry was then packed in a 5 ml column and the flow-through collected. The column was washed twice with 4 ml of wash buffer (50 mM $NaH_2PO_4$, pH 8.0, 300 mM NaCl, 20 mM imidazole), and the fusion protein was then eluted with imidazole (250 mM in wash buffer). Seven 0.5 ml elution fractions were collected and stored at −20 °C until analysis.

### 2.4. IL-2 bioassay and neutralisation

Thymic lobes were removed from 5 to 6 week old birds *post mortem* and were immediately placed in DMEM. Using blunt forceps, tissues were then teased apart in 10 ml of DMEM, resulting in a single cell suspension. The thymocytes were then layered over Ficoll-Paque (Amersham Pharmacia Biotech, St. Albans, UK), centrifuged at 1500*g* for 20 min and resuspended at $2 \times 10^7$ cells/ml in DMEM containing 2 mg/ml BSA, 1% L-glutamine and P/S, in the presence of 8 μg/ml PHA. This cell suspension (100 μl) was added to round-bottomed 96-well plates containing serial two-fold dilutions of recombinant (r) ChIL-2 (ex-COS), in a final volume of 200 μl/well. Negative controls included serial two-fold dilutions of supernatant collected from COS-7 cells transfected with pCI-neo alone, or media alone. Assays were carried out in triplicate. Cells were pulsed with $^3$H-thymidine (37 kBq/well, NEN, DuPont) for the final 6 h of a 48 h incubation at 41 °C, 5% $CO_2$. $^3$H-thymidine uptake was determined by liquid scintillation.

For the neutralisation assay, samples were pre-incubated with the mouse anti-ChIL-2 MAbs described in this paper, or irrelevant (anti-bovine) isotype-matched control MAbs (CC51 (IgG2b) or ILA-57 (IgG2a)), in a 1:2.5 dilution of TCF, for 1 h at RT, before addition of the IL-2-responsive cells.

### 2.5. Genetic immunisation

Two BALB/c mice were injected up to four times, every 4 weeks with 100 μg of DNA (pCI-neo-ChIL-2), in a total volume of 100 μl PBS. Fifty microlitres were injected into each hind quadriceps muscles. Two weeks after the second and third plasmid inoculations, sera were recovered and their titre tested in indirect ELISA (data not shown).

### 2.6. Hybridoma production

Five days prior to fusion, the mice were injected intraperitoneally with 1 ml of rChIL-2 ex-COS. Spleen cells were fused with Sp2/0-Ag14 mouse myeloma cells (Shulman et al., 1978) according to standard procedures (Galfre et al., 1977). The fused cells were plated out in 96-well tissue culture plates in 200 μl/well of RPMI (Life Technologies) containing 10% FBS, 10% hybridoma cloning factor (Origen, Gaithersburg, MD), 1 U/ml penicillin and 1 μg/ml streptomycin. Supernatants from all wells were screened for antibodies to ChIL-2 using an indirect ELISA described below. Positive hybridomas were selected for cloning (by limiting dilution) and expansion. The isotype of each MAb was determined using the indirect ELISA, with isotype-specific secondary MAbs, and confirmed using the IsoStrip mouse MAb isotyping kit (Boehringer Mannheim), according to manufacturer's instructions.

### 2.7. Antibody assays

Indirect ELISA was performed as follows. Falcon Microtest III flexible assay plates (Becton Dickinson, Oxnard, CA) were coated by overnight incubation at 4 °C with 50 μl of a 1:2 dilution of rChIL-2 ex-COS in 0.5 M carbonate buffer, pH 9.6. The plates were then washed three times in PBS containing 0.05% Tween-20 (PBS-T). Supernatant from fusion wells (50 μl) or 50 μl of mouse serum diluted 1:200 in PBS-T were added to the wells and incubated for 1 h at RT. After washing three times in PBS-T, 50 μl/well of biotinylated goat anti-mouse IgG (Amersham Pharmacia Biotech), diluted 1:1000 in PBS-T, were added with incubation for 1 h at RT. Binding of antibodies was detected by the addition of 50 μl/well of streptavidin-horseradish peroxidase (Amersham Pharmacia Biotech) diluted 1:500 in PBS with incubation for 1 h at RT. After washing three times in PBS-T, peroxidase activity was revealed by adding 75 μl/well of *o*-phenylenediamine (OPD) (4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0) substrate (Sigma, Poole, UK) for 10–15 min at RT. The reaction was stopped by the addition of 75 μl/well of 2 M $H_2SO_4$. Absorbance was read at 492 nm in a Spectra Max 250 microplate spectrophotometer system (Molecular Devices, Sunnyvale, CA).

Dot blots were performed as follows. Samples were dotted onto nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) and allowed to air-dry. Blots were then incubated in blocking solution (5% skimmed milk powder in PBS) overnight, at RT. After washing three times in PBS-T, they were incubated with the anti-ChIL-2 MAbs, diluted 1:10 in blocking buffer, for 1 h at RT. Primary antibody was removed by washing three times in PBS-T and blots were then incubated with rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase (DAKO, Denmark) diluted 1:1000 in PBS, for 1 h at RT. After a further five washes in PBS-T, detection was carried out using enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech), according to manufacturer's instructions.

Western blotting was performed as follows. Fifteen millilitre samples of rChIL-2 and rTuIL-2 (ex-COS) and "neo" (ex-COS) supernatants were concentrated to 0.7 ml using Centriprep concentrators (Amicon, Beverly, MA) according to manufacturer's instructions. SDS-PAGE analysis was according to Laemmli (1970) and gels were either stained with

Coomassie Brilliant Blue (Sigma) (Sambrook et al., 1989) or Western blots (Sambrook et al., 1989) were onto nitrocellulose membrane (Schleicher & Schuell). Immunological detection of rChIL-2 using our anti-ChIL-2 MAbs was essentially as described for dot blot analysis.

### *2.8. Deglycosylation of rChIL-2 (ex-COS)*

Recombinant ChIL-2 expressed in COS-7 cells was enzymatically deglycosylated using a kit (Bio-Rad), following manufacturer's instructions. Concentrated rChIL-2 was diluted in Reaction Buffer (50 mM $NaH_2PO_4$, pH 6.0 (final concentration)) and treated with a combination of NANase II (0.02 U) and *O*-glycosidase (0.002 U) for 1 h at 37 °C, to remove all *O*-linked oligosaccharides. One sample of the protein was then denatured by the addition of SDS and β-ME (to final concentrations of 0.125% and 62.5 mM, respectively) and boiling for 5 min. It was then cooled on ice for 5 min and NP-40 was added to a final concentration of 6%. Both denatured and non-denatured protein samples were then digested with PNGase F (0.005 U) for 3 h at 37 °C, to remove all *N*-linked oligosaccharides.

### *2.9. Immunofluorescence staining*

Splenocytes from 6 week old Sykes RIR were isolated (essentially as described above for thymocytes), and cultured in DMEM/BSA, in the presence of 1 μg/ml ConA and 10 μg/ml Brefeldin A (Sigma), at $10^6$ cells per well, in a final volume of 200 μl. Incubation was at 41 °C, 5% $CO_2$, for 4–5 h. Cells were then washed twice in PBS and fixed in 1% paraformaldehyde in PBS, for 10 min at RT. After washing twice in PBS, cells were then permeabilised in FACS permeabilising solution (Becton Dickinson) for 10 min at RT. Cells were then washed twice in PBS containing 1% w/v BSA and 0.1% w/v sodium azide (PBS/BSA/Az) and then incubated with 25 μl of anti-Ch IL-2 MAb (or appropriate isotype-matched control) for 30 min at RT. Cells were washed twice in PBS/BSA/Az and subsequently incubated with isotype-matched goat anti-mouse IgG conjugated to phycoerythrin (PE) (Southern Biotechnology Associates, Birmingham, AL) diluted 1:200 in PBS/BSA/Az for 20 min at RT. After two final washes in PBS/BSA/Az, cells were analysed on a FACScaliber flow cytometer (Becton Dickinson).

## 3. Results

### *3.1. Production and characterisation of MAbs to ChIL-2*

Pre-immune sera and test sera were collected from tail bleeds, to monitor Ab response by indirect ELISA (data not shown). Low to undetectable responses were seen for both mice after two DNA inoculations, from sera collected 2 weeks after the second injection. After the third injection, both mice showed an improved Ab response against the rChIL-2 antigen. Although neither of the titres was especially high, the best responder was given a final boost of rChIL-2 prior to fusion. The remaining mouse received a fourth injection. Ab titres in serum taken 2 weeks later showed little difference to those measured previously. The remaining mouse was then given a final boost of rChIL-2 protein, prior to fusion.

Fusions were carried out 5 days after the boost and wells which showed growth of hybridoma cells were screened for the production of antibodies to ChIL-2 by an indirect ELISA using rChIL-2 ex-COS as target antigen. From each fusion, one hybridoma secreted antibodies to ChIL-2. These were cloned by limiting dilution and the resulting MAbs were termed 10E7 and 4F12.

Isotype analysis of the MAbs produced by these clones revealed that 10E7 was IgG2b and 4F12 was IgG2a.

Both MAbs were tested for their reactivity to ChIL-2 in indirect ELISA, Western blotting and dot blots (Figs. 1 and 2). Both MAbs reacted with rChIL-2 ex-COS, but only

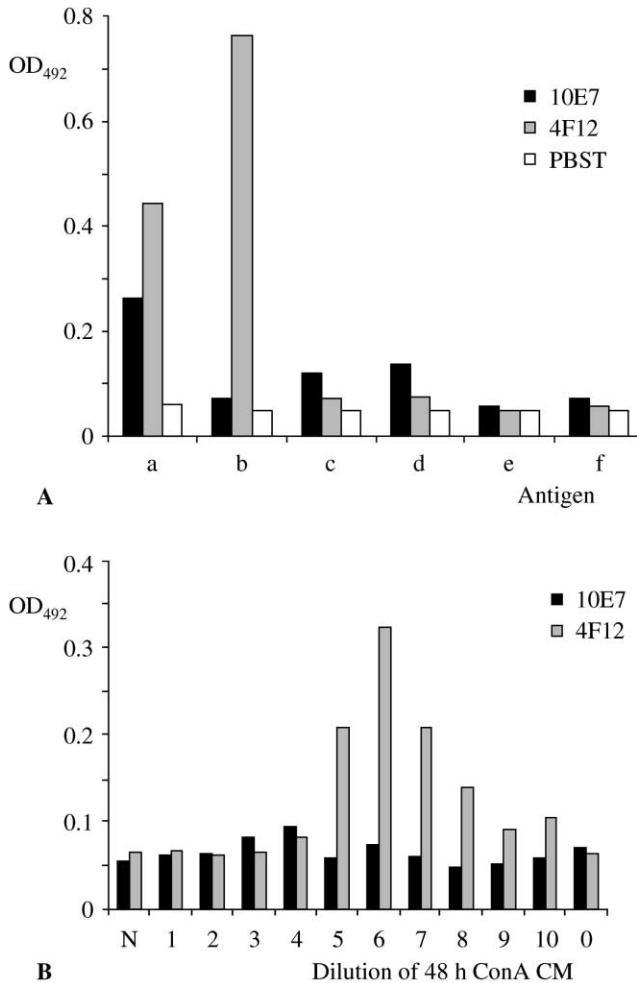


Fig. 1. Indirect ELISA results. (A) Cross-reactivity of anti-ChIL-2 MAbs against rIL-2 from various species and sources—a: rChIL-2 ex-COS; b: rChIL-2 ex-*E. coli*; c: rTuIL-2 ex-COS; d: rHuIL-2 ex-*E. coli*; e: rBoIL-2 ex-COS; f: no antigen. (B) Reactivity of anti-ChIL-2 MAbs with CM from 48 h ConA-stimulated chicken splenocytes—N: neat CM; 1–10: increasing two-fold dilutions of CM; 0: no CM (media only control).

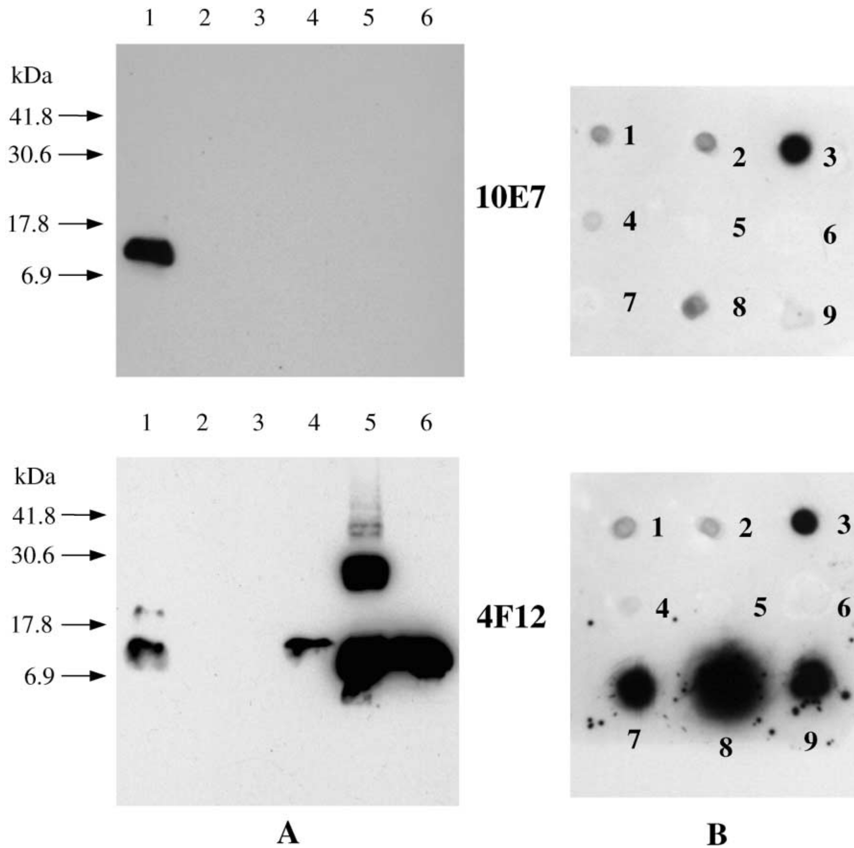


Fig. 2. (A) Western blots showing the specificity of anti-ChIL-2 MAbs 10E7 and 4F12. Secondary Ab was anti-mouse Ig-HRP and detection was by ECL—lane 1: rChIL-2 ex-COS; lane 2: rTuIL-2 ex-COS; lane 3: neo ex-COS (negative control); lane 4: purified rChIL-2 ex-*E. coli*; lane 5: rChIL-2 ex-*E. coli* (crude soluble fraction); lane 6: rChIL-2 ex-*E. coli* (crude insoluble fraction). (B) Dot blots showing the specificity of anti-ChIL-2 MAbs 10E7 and 4F12. Secondary Ab was anti-mouse Ig-HRP and detection was by ECL—1: neo ex-COS (negative control); 2: rChIFN-$\gamma$ ex-COS; 3: rChIL-2 ex-COS; 4: rTuIL-2 ex-COS; 5: rHuIL-2 ex-*E. coli*; 6: rBoIL-2 ex-BAC; 7: purified rChIL-2 ex-*E. coli*; 8: rChIL-2 ex-*E. coli* (crude soluble fraction); 9: rChIL-2 ex-*E. coli* (crude insoluble fraction).

4F12 reacted with rChIL-2 ex-*E. coli*. Neither of the MAbs reacted with rTuIL-2, rHuman (Hu) IL-2 or rBovine (Bo) IL-2.

Attempts to show specificity for native IL-2, assumed to be a significant component of conditioned media (CM) harvested from 48 h ConA-stimulated chicken splenocytes, indicated that only 4F12 recognised the native protein (Fig. 1B), and this was only apparent when the CM was diluted between $\frac{1}{32}$ and $\frac{1}{1024}$.

### 3.2. Deglycosylation of rChIL-2 (ex-COS)

Results from the indirect ELISA and immunoblotting analysis suggested that MAb 10E7 recognised a different epitope to that seen by MAb 4F12. Since 10E7 bound to rChIL-2

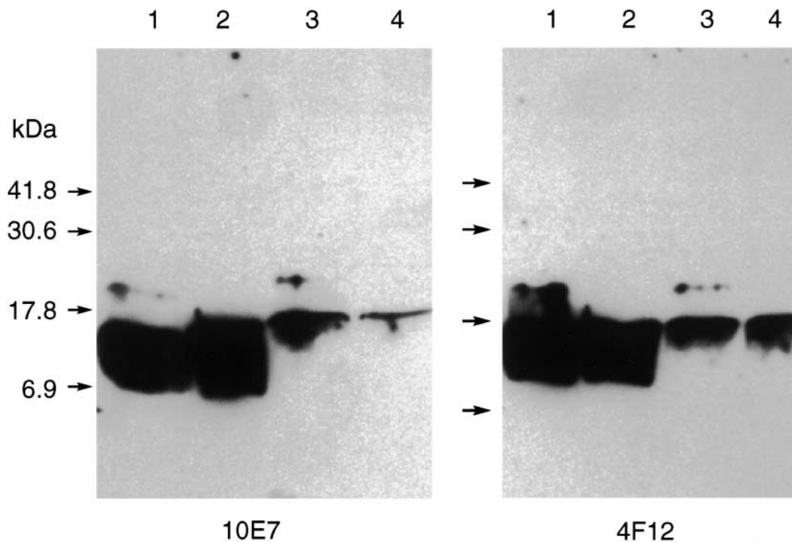


Fig. 3. Effect of deglycosylation of rChIL-2 (ex-COS) on the binding ability of anti-ChIL-2 MAbs. Analysis by Western blot and ECL detection, as described for Fig. 2A. Concentrated rChIL-2 (ex-COS) was used for all samples. Lane 1: pre-deglycosylation (non-denaturing conditions); lane 2: post-deglycosylation (non-denaturing conditions); lane 3: pre-deglycosylation (denaturing conditions); lane 4: post-deglycosylation (denaturing conditions).

derived from the mammalian COS expression system, but failed to bind to *E. coli*-derived rChIL-2, perhaps 10E7 recognises an epitope involved in glycosylation.

Identical samples of concentrated rChIL-2 (ex-COS) were digested with NANase II and *O*-glycosidase, followed by PNGase F, under non-denaturing or denaturing conditions. Entire samples were then analysed by SDS-PAGE and Western blot, and were probed with either 10E7 or 4F12. Two bands ($\sim$17 and $\sim$20 kDa) were detected by both MAbs in the control, pre-treated samples. After deglycosylation, the faint, larger band was no longer detected by either MAb (Fig. 3). Apart from this, no difference was seen in the binding capacity of 4F12 pre- and post-glycosidase treatment. However, 10E7 appeared to bind to less protein after glycosidase treatment (in denaturing conditions) when compared to untreated rChIL-2. Despite repeating this experiment, complete elimination of binding of 10E7 to its antigen was never demonstrated. Deglycosylation under non-denaturing conditions had little effect on the binding capacity of either 10E7 or 4F12.

### 3.3. Biological activity of MAbs

Purified MAbs were also assayed for their ability to inhibit the biological activity of rChIL-2, as measured with the thymocyte co-stimulation assay described in Section 2. Both MAbs were capable of neutralising the biological activity of rChIL-2 ex-COS (Fig. 4). When rChIL-2 was incubated with irrelevant (anti-bovine) isotype-matched control MAbs (CC51 for 10E7 or ILA-57 for 4F12), no significant reduction in proliferation was seen when compared to the positive control (rChIL-2 with no MAb added).

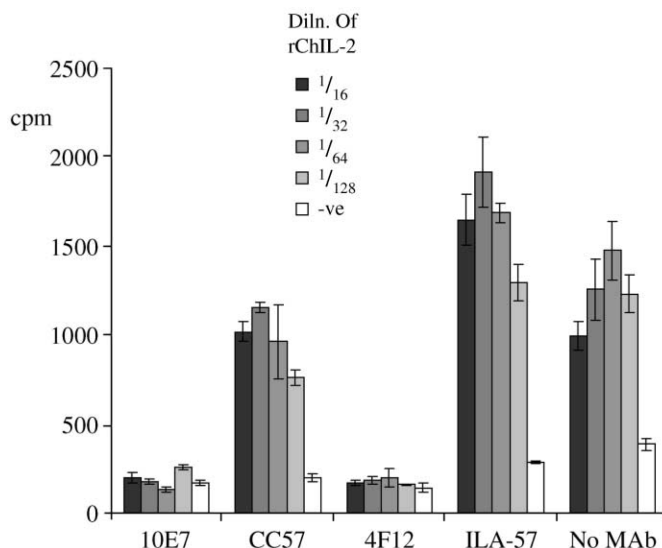


Fig. 4. IL-2 neutralising assay showing effect of anti-ChIL-2 MAbs on proliferative bioactivity of rChIL-2 (ex-COS) on sub-optimally stimulated thymocytes. Dilutions of rChIL-2 (ex-COS) were pre-incubated with anti-ChIL-2 MAbs 10E7 and 4F12, or with isotype-matched control MAbs CC51 and ILA-57. These samples were then incubated with thymocytes, in the presence of PHA, for 72 h. Proliferation was measured by incorporation of $^{3}$H-thymidine. Values represent the mean of triplicate wells. Error bars represent S.E. of the mean. Data shown is representative of four individual assays.

### 3.4. Intracellular staining

Chicken splenocytes were activated with ConA in the presence of Brefeldin A and stained for intracellular IL-2 with both MAbs. Neither MAb proved suitable for intracellular staining (data not shown).

## 4. Discussion

This paper describes the production and characterisation of the first MAbs specific for ChIL-2. The two anti-ChIL-2 MAbs produced in this study were shown to be specific for ChIL-2, by ELISA and also in dot blots and Western blots. No cross-reactivity was detected against either turkey or mammalian IL-2s using these techniques. It is not surprising that the anti-ChIL-2 MAbs did not recognise an epitope on mammalian IL-2, considering the low level of identity between chicken and mammalian IL-2s (e.g. 24% identity with HuIL-2 at the amino acid level) (Sundick and Gill-Dixon, 1997). However, there is a much higher degree of conservation between chicken and turkey IL-2 (70% aa identity), sufficient to enable the two avian proteins to cross-react in functional assays (Lawson et al., 2000). In spite of this, it seems both 10E7 and 4F12 recognise epitopes which include amino acid substitutions in the turkey molecule. Since the nucleotide

differences between the two avian sequences are not clustered in any particular region (Lawson et al., 2000), without epitope mapping experiments it is hard to predict where these epitopes may lie.

To show specificity of the MAbs for native ChIL-2, CM from 48 h ConA-stimulated splenocytes was used as a source of antigen for ELISA and immunoblots. Indirect ELISA results showed 4F12 bound to a component of the CM, when diluted between $\frac{1}{32}$ and $\frac{1}{1024}$. The CM contained a high concentration of BSA (2 mg/ml), which was possibly blocking binding of the MAb until diluted out sufficiently. Disappointingly, 10E7 failed to bind to antigen within CM in the indirect ELISA. There are several possible reasons for this, including the possibility that the conformation of the native molecule is slightly different to that of the recombinant protein, resulting in the appropriate epitope not being accessible to the MAb. Alternatively, the binding affinity of 10E7 may not be strong enough to overcome the blocking capacity of the BSA in the CM.

Both MAbs recognised the cytokine expressed in COS cells, but only 4F12 recognised ChIL-2 produced in *E. coli*. This suggests that 10E7 may recognise an epitope on rChIL-2 involved in glycosylation. Comparison of rChIL-2 derived from the two expression systems, when probed with 4F12 in a Western blot (Fig. 2A), indicates that there is little, if any, difference in migration, and hence size (both ~16–17 kDa), of the two recombinant proteins. However, it was noted in other Western blots that both MAbs almost always immunoblotted a second, larger band in rChIL-2 ex-COS (Figs. 2A and 3). This band may be attributed to a glycosylated form of the recombinant protein, suggesting that the protein expressed by the COS cells is not homogenous in structure. Indeed in HuIL-2, there is a variable degree of glycosylation, which is responsible for size and charge heterogeneity of the mature protein (Robb and Smith, 1981).

To test the hypothesis that ChIL-2 is a glycosylated protein, and that 10E7 binds to an epitope involved in glycosylation, rChIL-2 (ex-COS) was digested with enzymes which remove *N*- and *O*-linked oligosaccharides. Digestion appeared to remove the larger band (Fig. 3) when immunoblotted with either MAb, evidence that the larger band represents glycosylated rChIL-2. Enzymatic treatment also appeared to reduce the staining intensity of 10E7, but binding was never completely abolished after digestion. It is possible that deglycosylation was incomplete, which may account for the reduction in, but not removal of, the binding capacity of 10E7, if the proposed hypothesis is true. The combination of NANase II and *O*-glycosidase does not necessarily remove all *O*-linked sugars, so additional enzymes may be needed to ensure complete deglycosylation.

Both MAbs generated in this project can neutralise the proliferative activity of rChIL-2, as measured in a thymocyte co-stimulation assay. Results were clear-cut and conclusive over a range of dilutions of rChIL-2 and the neutralising effect titrated out with increasing dilution of MAb. Further evidence of the blocking ability of these MAbs has been demonstrated in a collaboration investigating the effect of rChIL-2 on chicken heterophils (Kogut et al., 2001). The neutralising ability of both MAbs on "native" ChIL-2 found in CM was also investigated (data not shown). Results supported data discussed earlier, which showed only 4F12 bound to ChIL-2 in CM. In the neutralising assay, pre-incubation with 10E7 resulted in slight, but insignificant inhibition of CM-induced proliferation, whereas 4F12 resulted in a greater degree of inhibition of the proliferative activity. One would not necessarily expect all the proliferative activity found within CM to be blocked by

anti-ChIL-2 MAbs, since it is to be expected that after mitogen stimulation, other proliferative cytokines may have been secreted into the culture media.

Unsuccessful attempts were made to stain intracellular ChIL-2 expressed in transfected COS-7 cells, as a prelude to intracellular staining of cytokines in tissues. More extensive experiments were carried out to assess the application of 10E7 and 4F12 in intracellular flow cytometry. If suitable, these MAbs would be extremely useful in helping to identify which cell types express ChIL-2, and in conjunction with other suitable anti-chicken cytokine MAbs (when they become available), would enable some progress in identifying T1-like cells in the chicken for the first time. However, detailed, but inexhaustible studies indicated that neither of the MAbs described in this study were successful candidates for use in flow cytometry. Although both MAbs at first appeared to identify cellular antigens after stimulation, fixation and permeabilisation, which were at least partially blocked by incubation with rChIL-2, controls indicated that staining was not specific.

To conclude, the MAbs produced in this study both recognise linear epitopes in the ChIL-2 molecule, although MAb 10E7 seems to be limited to only binding rChIL-2 ex-COS. 4F12, on the other hand, recognises both the glycosylated protein and the unmodified recombinant molecule produced by bacterial cells, as well as native IL-2 found within CM. This particular pair of MAbs is not compatible with the fixation and permeabilisation procedure required for immunofluorescent staining of intracellular antigens. Both MAbs are, however, capable of neutralising the bioactivity of rChIL-2. We are currently investigating the potential of these MAbs for use in an IL-2 capture ELISA.

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