Protein Engineering, Design & Selection vol. 21 no. 5 pp. 283–288, 2008
Published online April 2, 2008 doi:10.1093/protein/gzm067

Anti-serum albumin domain antibodies for extending the half-lives of short lived drugs

Lucy J. Holt[1], Amrik Basran, Kate Jones, Jennifer Chorlton, Laurent S. Jespers, Neil D. Brewis and Ian M. Tomlinson

Domantis Limited, 315 Cambridge Science Park, Cambridge, CB4 0WG, UK

[1]To whom correspondence should be addressed. lucy.holt@domantis.com

We have used phage display to isolate a range of human domain antibodies (dAbs) that bind to mouse, rat and/or human serum albumin (SA) and can be expressed at very high levels in bacterial, yeast or mammalian cell culture. In contrast to non-SA-binding dAbs, which have terminal half-lives of less than 45 min, the half-lives of these 12 kDa 'AlbudAbs' can match the half-life of SA itself. To demonstrate the use of AlbudAbs for extending the half-lives of therapeutic drugs, we created a fusion of the interleukin-1 receptor antagonist (IL-1ra) with an AlbudAb. Soluble IL-1ra is potent inhibitor of IL-1 signalling that is approved for the treatment of rheumatoid arthritis but has a relatively short *in vivo* half-life. Here we show that although the AlbudAb/IL-1ra fusion has a similar *in vitro* potency, its *in vivo* efficacy can be dramatically improved due to its extended serum half-life. AlbudAbs could potentially be used to generate a range of long half-life versions of many different drugs in order to improve their dosing regimen and/or clinical effect.
Keywords: AlbudAb/albumin/domain antibody/half-life/dAb

Introduction

Recombinant human proteins such as interferon alpha and beta, growth hormone, insulin, erythropoietin and granulocyte/granulocyte-macrophage colony stimulating factors (G-CSF and GM-CSF) are major drugs that have been at the forefront of the biotechnology revolution. In their natural state, most of these proteins are subject to rapid turn over, meaning that they are both synthesized and degraded/excreted relatively quickly. Unfortunately, this means that, as therapeutic drugs, they need to be frequently injected in order to keep up with the natural processes of clearance. This has led to the development of several approaches that can be used to extend the dosing interval of drugs. While some of these rely on specific slow release formulations (Mainardes and Silva, 2004) or reduction of susceptibility to serum proteases (Werle and Bernkop-Schnürch, 2006) others aim to reduce the drug's intrinsic rate of clearance by amino acid substitutions that reduce receptor binding affinity in intracellular endosomal compartments, thereby leading to increased recycling in the ligand-sorting process and consequently resulting in longer half-life in extracellular medium (Sarkar *et al*., 2002). A second widely used approach to reduce rate of clearance is direct conjugation to a second molecule that has an inherently long serum half-life. One such method is to increase the hydrodynamic size of the protein by chemical attachment of polyethlylene glycol (PEG) (Chapman, 2002; Pockros *et al*., 2004; Veronese and Pasut, 2005), which can produce a drug with a terminal half-life in humans of up to 14 days (Choy *et al*., 2002). A second approach is to express the therapeutic protein as a genetic fusion with a natural protein that has a long serum half-life; either 67 kDa serum albumin (SA) (Syed *et al*., 1997; Osborn *et al*., 2002) or the Fc portion of an antibody, which adds an additional 60–70 kDa in its natural dimeric form, depending on glycosylation (Mohler *et al*., 1993). This yields drugs that have terminal half-lives in humans of several days [e.g. 5–8 days for interferon alpha fused to albumin (http://www.hgsi.com) and 4 days for TNF receptor (p75) fused to an Fc region (Lee *et al*., 2003)].

Here we wondered whether we could use human Domain Antibodies (dAbs) (Ward *et al*., 1989) that bind to SA (which we have termed 'AlbudAbs') as genetic fusions to increase the *in vivo* half-life of recombinant proteins. Albumin was chosen as a target because it has a serum half-life in humans of 19 days (Peters, 1985), is a carrier protein for various hormones and other natural molecules and is a well-validated carrier for many small molecule drugs. Albumin is also known to accumulate in tumors (Jain, 1988) and in arthritic joints (Wunder *et al*., 2003), potentially enabling a further level of targeting in disease specific situations. Natural bacterial proteins that bind to both albumin and immunoglobulins were described in 1988 (Sjobring *et al*., 1988), but their foreign sequence could raise concerns regarding immunogenicity. Fifty-two kilodalton Fab fragments that bind to rat SA and 'diabodies' composed of four immunoglobulin V domains expressed as two chains that bind to IgG have previously been shown to increase the half-life of chemically conjugated proteins and antibody fragments, respectively (Holliger *et al*., 1997; Smith *et al*., 2001), but such molecules are relatively large and complex to express at a pharmaceutical-scale. Albumin binding peptides have previously been shown to extend serum half-life of a therapeutic Fab (Dennis *et al*., 2002; Nguyen *et al*., 2006) but do not achieve the half-life of albumin itself (perhaps due to protease susceptibility). dAbs on the other hand are small (11–13 kDa in size), highly stable and well expressed proteins that have fully human scaffolds (Jespers *et al*., 2004a, b). They should therefore make ideal fusion partners for creation of therapeutic proteins with long serum half-lives.

Methods

Phage display selection of human V_H and V_κ dAb libraries

Phage from large synthetic dAb repertoires [1.6×10^{10} for V_H (Jespers *et al*., 2004a) and 1.7×10^{10} for V_κ] based on two human frameworks comprising the heavy-chain germline genes V3-23/DP47 and JH4b for the V_H library and the κ light chain genes O12/O2/DPK9 and Jκ1 for the V_κ library

with side chain diversity incorporated at positions in the antigen binding site were prepared and purified as decribed (McCafferty *et al*., 1990). dAbs were selected against biotinylated SA in solution or SA coated on an immunotube (Nunc) or a combination of the two methods. Immunotubes were coated overnight with SA at 10 μg/ml and blocked with 2% Marvel PBS (MPBS). Purified phage were diluted with MPBS and incubated for 2 h in the immunotubes. For biotinylated selections, 50–200 nM antigen was incubated for 1 h with 100 μl phage in MPBS and then captured for 10 min on streptavidin Dynabeads (Dynal, UK). In both soluble and tube selections, after 10 washes (for the first round) or 20 washes (for subsequent rounds), SA-bound phage were eluted in 500 μl of 100 μg/ml trypsin in PBS for 10 min and then used to infect 1.75 ml of log-phase *Escherichia coli* TG1 for 30 min. Serial dilutions (for phage titer) and library plating were using an appropriate antibiotic. For subsequent rounds of selection, cells were scraped from the plates and used to inoculate 50 ml cultures that were grown overnight at 37°C for phage amplification.

Expression of AlbudAbs, dAb/IL-1ra fusions and IL-1ra

The genes encoding selected dAbs were cloned as polyclonal selection outputs into a pUC based ampicillin resistant vector with a C-terminal myc tag. After transformation into *E.coli*, and picking individual colonies, small-scale dAb expression was induced in 96-well plates in the presence of 1 mM IPTG (final concentration) at 30°C for 16 h. After centrifugation, the supernatants were tested for binding to SA in ELISA. Larger scale expression, with the myc tag replaced by an HA tag, was performed using the OnEx lactose-based induction system (Novagen). Purification was via protein A sepharose (for V_H dAbs) and protein L-agarose (for V_κ dAbs). dAb/IL-1ra fusions were expressed over 4 h by induction with IPTG in a tagless pET-12a based vector system in *E.coli* BL21 DE3 PlysS. Protein was recovered from the periplasm by osmotic shock and purified by protein L affinity chromatography followed by loading onto an anion exchange resource-Q column (Akta) in 20 mM Tris pH9 and elution with a linear gradient of 0.5M NaCl, 20 mM Tris over 10 column volumes. Fractions containing the correct protein were identified by SDS–PAGE and pooled. IL-1ra was expressed in a tagless pET-based vector system followed by four step purification involving acid precipitation at pH 4.5 and anion exchange at pH 8 both to remove *E.coli* proteins, followed by cation exchange at pH 4 and gel filtration [modified from (Shuck *et al*., 1991)].

In vitro assays of protein activity

For initial identification of dAbs that bind SA, ELISA wells were coated overnight at 4°C with SA at 10 μg/ml in PBS. After blocking the wells with 2% tween PBS (PBST), dAb in PBST was incubated for 1 h. After washing, two step detection was with anti-myc antibody 9E10 and peroxidase-conjugated rabbit anti-mouse antibody using 3,3′,5,5′,-tetramethylbenzidine as substrate. For analysis of dAb affinity for SA, 500 resonance units of albumin were coupled to a CM5 Biacore chip at pH 5.5 and binding curves generated by flowing dAb diluted in Biacore HBS-EP buffer at a range of concentrations of dAb in the range 5 nM to 5 mM across the Biacore chip. Affinity (K_D) was calculated by fitting on-rate and off-rate curves for traces generated in the range of the kDa for each dAb. For analysis of IL-1ra activity, proteins were tested for the ability to neutralise the induction of IL-8 secretion by IL-1 in MRC-5 cells [method adapted from that of Akeson, L. *et al*. (Akeson *et al*., 1996) describing the induction of IL-8 by IL-1 in HUVEC; here we use MRC-5 cells instead of the HUVEC cell line]. Briefly, MRC-5 cells plated in microtitre plates were incubated overnight with dAb and 100 pg/ml IL-1α. Post-incubation the supernatant was aspirated off the cells and IL-8 concentration measured with a sandwich ELISA (R&D Systems). Stability of IL-1ra in the serum was assessed by incubation of 100 μg/ml IL-1ra or dAbm16/IL-1ra in 0.03% azide, 1x mouse serum for 36 h at 37°C in a humid environment or as a control at −20°C. Analysis of samples was by Western plot of an SDS–PAGE gel probed with polyclonal rabbit anti IL-1ra (Abcam, UK) and then goat anti-rabbit HRP conjugate followed by development with ECL+ (Amersham Biosciences, UK).

Pharmacokinetics and biodistribution of dAbs in mouse and rat

Groups of 24 mice received an intravenous bolus of 1–2 mg/kg of dAbm16HA, irrelevant dAb, dAbm16/IL-1ra, irrelevant dAb/IL-1ra or IL-1ra and serum samples obtained from terminal bleeds of groups of three mice over a 7 day period were analysed by anti HA, protein L sandwich ELISA or an anti IL-1ra sandwich ELISA using antibodies against two different IL-1ra epitopes (R&D Systems). Serum concentration versus time curves were fitted for a 1 compartment model using WinNonlin software (IL-1ra and irrelevant dAb) and a 2 compartment model using Kaleidograph software (dAbm16HA). Groups of four to five rats received an intravenous bolus of 1–3 mg/Kg of ^{3}H RSA, ^{3}H dAbr3 or ^{3}H dAbr16 and serum samples were obtained over a 7 day period. Serum concentration versus time curves were fitted for a 2 compartment model using Kaleidograph software. For whole body autoradiography, mice received an intravenous bolus of ^{3}H MSA, ^{3}H dABh8/IL-1ra or ^{3}H IL-1ra at 2–3 mg/kg and after 1 h animals were humanely sacrificed followed by immediate snap freezing in hexane cooled to −80°C with solid carbon dioxide. Each frozen carcass was set in a block of 1% (w/v) aqueous carboxymethylcellulose and sagittal sections (nominally 30 μm) taken using a CM3600 cryomicrotome (Leica Microsystems). Sections were freeze dried and placed in contact with imaging plates (type BAS-III, Raytek Scientific).

Efficacy of AlbudAb/IL-1ra fusion in mouse collagen induced arthritis model of rheumatoid arthritis

DBA/1 mice were injected once with an emulsion of Arthrogen-CIA adjuvant and Arthrogen-CIA collagen. At day 21, animals were allocated into groups of 10 and treated three times a week from day 21 to 49 with an intraperitoneal bolus of IL-1ra or dAbm16/IL-1ra at two doses of 1 mg/kg and 10 mg/kg. Control groups were treated with saline and a subcutaneously administered 0.4 mg/kg positive control steroid, dexamethasone. Body weight and clinical scores for the severity of arthritis (see figure legend) were recorded three times a week from day 21 to 49. Animals were scored at each treatment for severity of arthritis in each limb using the following scale: 0 normal, 1 mild but definite redness and swelling of the ankle or wrist, or apparent redness and

swelling limited to individual digits, 2 moderate redness and swelling of the ankle and wrist, 3 severe redness and swelling of the entire paw including digits and 4 maximally inflamed limb with involvement of multiple joints. Scores for the four limbs were summed and each animal given the resulting score (between 0 and 16). Individual animals were removed from the study for ethical reasons if the score was greater than 12. The experiment ended at day 49 for all animals or earlier for individual animals if necessary for ethical reasons. Using a statistically conservative score of 16 for any animals removed from the study, the day 49 arthritis scores and the area under the curve (a measurement of the severity of arthritis throughout the study) were statistically compared using a rank-based ANOVA.

Results

Selection and preliminary characterisation of AlbudAbs

A range of human V_H and V_κ dAbs were selected for binding to SA by phage display (Winter *et al*., 1994) from a series of large synthetic dAb libraries. These libraries are based on fully human germline scaffolds with targeted diversification of a subset of hypervariable loop residues creating sufficient structural diversity to derive binders to almost any target antigen (Jespers *et al*., 2004a). The *in vitro* isolation of dAbs to SA circumvents any problems that might be encountered in isolating anti-self specificities by immunisation and enables human dAb sequences (highly homologous to their germline V gene precursors) to be selected against SA from several different species, including humans. Accordingly, two or three rounds of selection were performed using rat serum albumin (RSA), human serum albumin (HSA) or alternating rounds using two or more of RSA, HSA, and mouse serum albumin (MSA). *Escherichia coli* expressed dAbs were tested for binding SA in ELISA and using surface plasmon resonance on Biacore. A collection of 37 human dAbs with different amino acid sequences were identified (Fig. 1), each with differing degrees of species cross reactivity (dAbh8, a V_κ) binds RSA, HSA and MSA, with affinities in the 34–600 nM range, making it ideal for both pre-clinical and clinical evaluation. Test expression in a shake-flask gave a yield of 310 mg/l in *E.coli*. A second V_κ domain, dAbm16, with an affinity of 70 nM for MSA was subjected to pharmacokinetic analysis in mice. Its terminal half-life (24 h) and

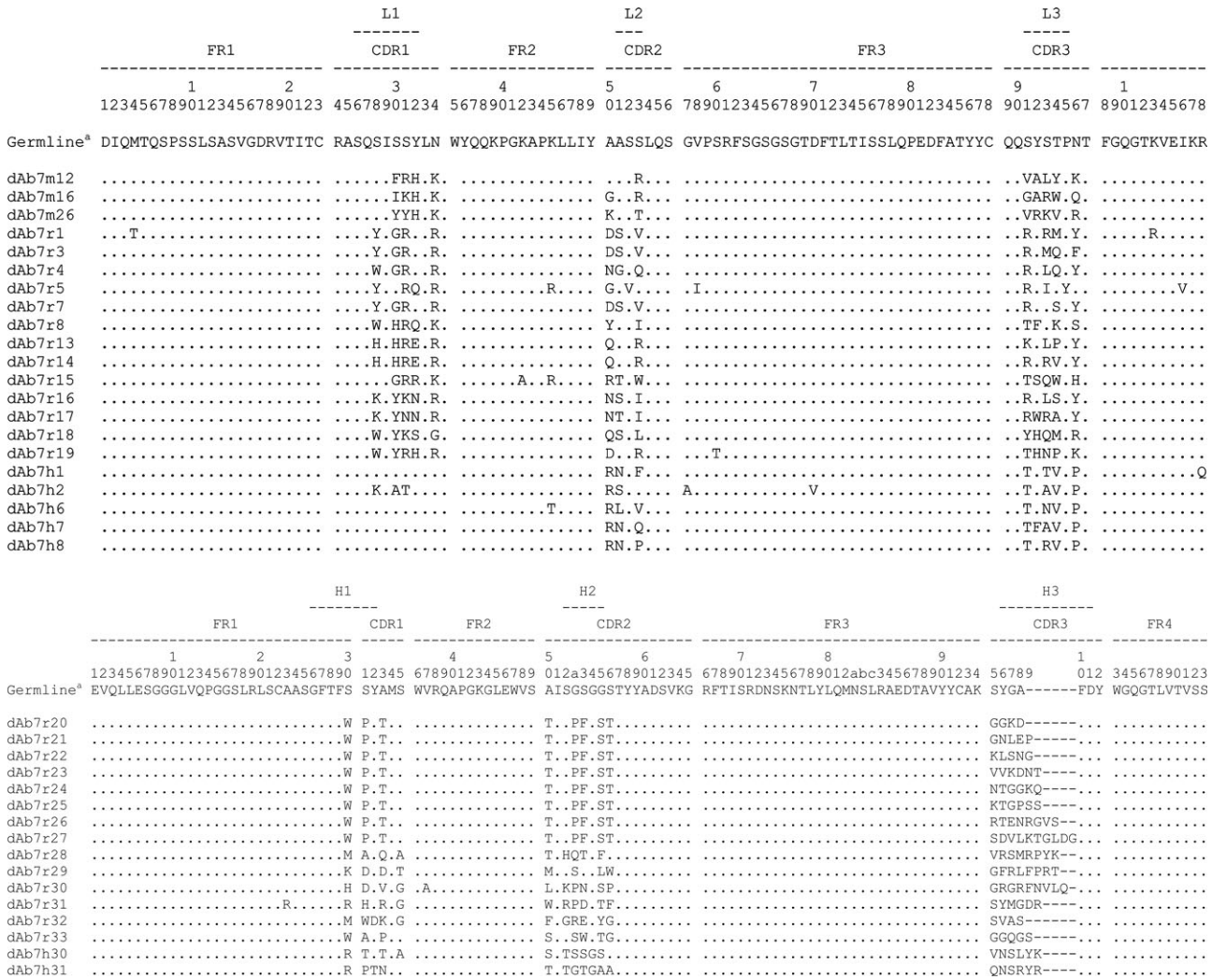

Fig. 1. Antibody sequences of AlbudAb clones identified by phage selection. [a]All clones have been aligned to the human germline genes. Residues that are identical to germline have been represented by'.'. In the V_H CDR3, the symbol '–' has been used to facilitate alignment but does not represent a residue. All clones were selected from libraries based on a single human framework comprising the heavy-chain germline genes V3-23/DP47 and JH4b for the V_H libraries and the κ light chain genes O12/O2/DPK9 and Jκ1 for the V_κ libraries with side chain diversity incorporated at positions in the antigen binding site.

area under the curve (AUC, 900 μg.h/ml) were significantly greater than similar measurements for a dAb that does not bind SA (42 min and 5 μg h/ml), representing an improvement in terminal half-life of 51-fold and in AUC of over 100-fold (Fig. 2A). Two further V_κ domains, dAbr3 and dAbr16 with affinities for RSA of 13 nM and 1 μM, respectively, were subjected to pharmacokinetic analyses in rats using RSA as a control. The three proteins were found to have half-lives of 53 h (dAbr3), 43 h (dAbr16) and 53 h (RSA) and AUCs of 1300 h μg/ml, 1000 h μg/ml and 2000 h μg/ml, respectively (Fig. 2B).

Production of AlbudAb fusions with IL-1ra

Two AlbudAbs (dAbm16 and dAbh8) with MSA reactivity were then used to create genetic fusions with the human form of the IL-1 receptor antagonist (IL-1ra), which is a naturally occurring inhibitor of IL-1 signalling. Although sub-cutaneous injection of recombinant IL-1ra is a proven therapy for rheumatoid arthritis (Jiang *et al*., 2000), it has a very short serum half-life in man meaning that daily sub-cutaneous dosing of 100 mg is required to maintain a therapeutic effect. The human form of IL-1ra is known to be active in both humans and mice, meaning that its *in vivo* efficacy can be assessed using a murine model of collagen induced arthritis (CIA) mouse (Courtenay *et al*., 1980; Brand *et al*., 2004).

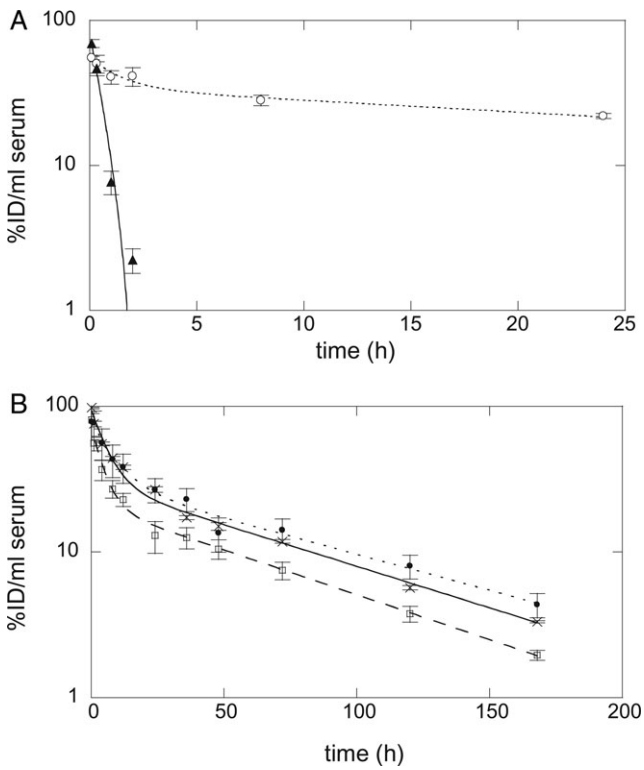

Fig. 2. Pharmacokinetics of AlbudAbs. (**A**) Two dAbs were injected separately into mice at 1 mg/kg and serum analysed at six timepoints with $n = 3$ at each timepoint. AlbudAb dAbm16 - - - -○- - - - has a half-life in mouse of 24 h and AUC of 900 μg h/ml and a dAb that binds an irrelevant antigen —▲— has a half life of 42 min and AUC of 5 μg h/ml. Serum samples were analysed by protein L/anti-HA tag ELISA and compared to a standard of known concentration. (**B**) Two dAbs and RSA were labelled with ^{3}H and separately injected into rats at 3 mg/kg and serum analysed at nine timepoints with $n = 4$ at each timepoint. AlbudAbs dAbr3 - - -●- - -, dAbr16 —□— and RSA —╳— have half-lives in rat of 53 h, 43 h and 53 h respectively and AUCs of 1300 h μg/ml, 1000 h μg/ml and 2000 h μg/ml respectively. Serum samples were analysed by scintillation counting.

Initially, dAbm16 was produced as both an N-terminal and C-terminal fusion with IL-1ra, but better processing and IL-1ra activity was observed when the dAb was N-terminal (data not shown) and so this format was adopted for all later experiments. Both the dAbm16/IL-1ra and the dAbh8/IL-1ra fusions were expressed in *E.coli* (data for dAbm16/IL-1ra shown in Fig. 3A) and tested *in vitro* for SA binding on Biacore and for IL-1ra activity by testing their ability to neutralise the induction of IL-8 secretion by IL-1 alpha in MRC-5 cells (Akeson *et al*., 1996). For both AlbudAb-IL-1ra fusions, the affinity for SA and IL-1ra activity was largely unaffected by the presence of a fusion partner, with the ND_{50} in the cell-based assay being 89, 110 and 200 pM for the IL-1ra alone, dAbm16/IL-1ra and dAbh8/IL-1ra, respectively (data for dAbm16/IL-1ra shown in Fig. 3B).

Stability and pharmacokinetics

Next, dAbm16/IL-1ra was subjected to an *ex vivo* serum stability analysis by immunoblot analysis and then to pharmacokinetic analyses in mice to test the activity of the SA-binding dAb *in vivo*. The fusion remained over 90% intact when incubated at 37°C in mouse serum for 1.5 days (data not shown) and the *in vivo* half-life ($t_{1/2\beta} = 4.3$ h; AUC = 270 h μg/ml) was significantly longer than for IL-1ra alone ($t_{1/2\beta} = 2$ min; AUC = 1.7 h μg/ml) or for the IL-1ra fused to an irrelevant (non-SA binding) dAb ($t_{1/2\beta} = 24$ min; AUC = 1.5 h μg/ml). Interestingly, the half-life of the fusion was not as long as for either AlbudAb alone or for AlbudAbs fused to other therapeutic moieties, even ones that bind cell surface receptors (unpublished data), indicating the some other mechanism specific to the IL-1ra part of the fusion may be mediating the apparent clearance of the fusion protein (or its constituent parts) from the bloodstream. Nevertheless, the area under the curve for the dAbm16/IL-1ra fusion was still over a 100-fold greater than for the fusion to the irrelevant dAb or for IL-1ra alone. This increase is of the same order of magnitude as that seen with other drugs fused directly to HSA (Halpern *et al*., 2002; Wunder *et al*., 2003).

Biodistribution in mice

In order to compare biodistribution of IL-1ra before and after fusion to the AlbudAb, we next administered IL-1ra alone, dAbh8/IL-1ra and MSA, each labelled with ^{3}H, to BALBc mice. Mice were sacrificed after 1 h, sectioned and imaged using a phosphorimager (Fig. 3C). In the absence of an AlbudAb fusion partner, IL-1ra is primarily located in the renal cortex [Fig. 3C(i)] and in the bladder [Fig. 3C(ii)], confirming rapid removal from the blood by glomerular filtration and excretion, whereas as a fusion to an AlbudAb [Fig. 3C(iii)], IL1-ra adopts a pattern of tissue distribution that is almost identical to that of MSA [Fig. 3C(iv)], demonstrating clear targeting of the AlbudAb.

Efficacy in mouse model of arthritis

We next compared the efficacy of the dAbm16/IL-1ra fusion with the efficacy of IL-1ra alone in the collagen induced arthritis model. At day 21 after collagen injection, animals were allocated into groups of 10 and treated three times a week for 4 weeks with an interperitoneal bolus of IL-1ra or

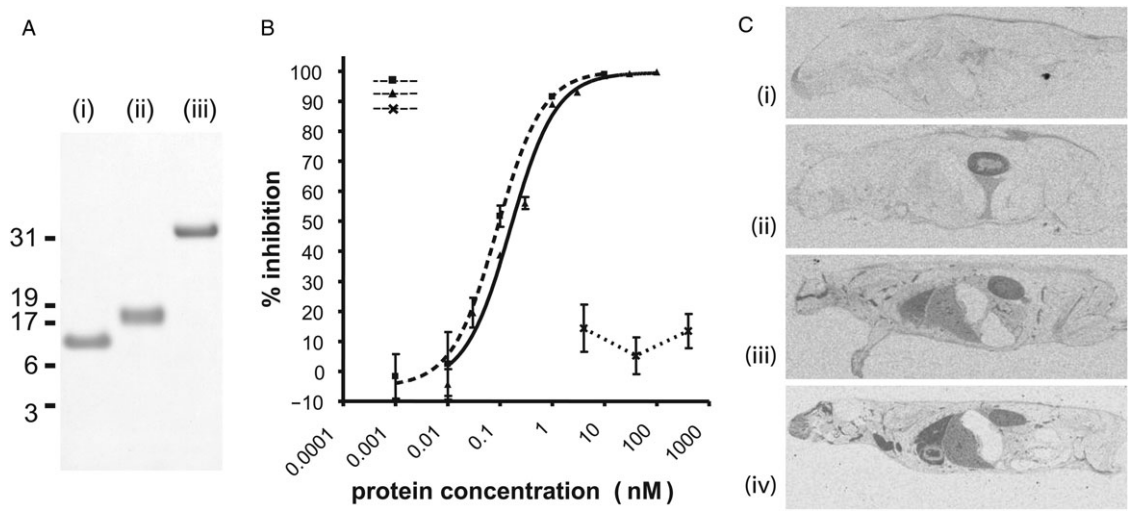

Fig. 3. Expression and *in vitro* activity of an AlbudAb fusion. (**A**) Coomassie stained gel of (i) dAbm16 alone, (ii) IL-1ra and (iii) dAbm16/IL-1ra fusion. (**B**) For analysis of IL-1ra activity, proteins were tested for the ability to neutralise the induction of IL-8 secretion by IL-1a in MRC-5 cells. IL-1ra — ■ — and the dAbm16/IL-1ra fusion —▲— neutralised IL-1a activity with an ND_{50} of 89 pM and 110 pM respectively. dAbm16 alone — ✕ — had no activity. (**C**) Quantitative whole body autoradiography 1 h after administration of (i and ii) IL-1ra alone, (iii) dAbh8/IL-1ra fusion and (iv) MSA. The biodistribution of dAb7h8/IL-1ra is similar to MSA and different to IL-1ra alone.

dAbm16/IL-1ra, each at doses of 1 mg/kg (Fig. 4A) and 10 mg/kg (Fig. 4B). Using clinical scores for the severity of arthritis (see Methods), we demonstrated that on day 49 at the end of the study, the mean arthritis score in the 1 mg/kg AlbudAb fusion group (4.5) was lower than the mean arthritis score in the 1 mg/kg IL-1ra group (8.7). Furthermore, the mean arthritis score in the 10 mg/kg AlbudAb fusion group (2.0) was lower than the mean arthritis score in the 10 mg/kg IL-1ra group (5.5). Using a rank-based ANOVA to compare these four treatment groups, both higher dose ($P < 0.05$) and addition of the AlbudAb ($P < 0.05$) were significant in lowering the day 49 arthritis score. Using AUC (a measurement of the severity of arthritis throughout the study) as an alternative method for assessing treatment efficacy, the same four treatment groups were compared in a rank-based ANOVA and both dose ($P < 0.05$) and addition of the AlbudAb ($P < 0.005$) were again significant. Thus, AlbudAbs can be linked to IL-1ra (a clinically proven therapy for RA) to create a fusion which *in vivo* exhibits both long serum half-life properties (conferred by the AlbudAb) and potent inhibition of IL-1 signalling (conferred by the IL-1ra). The greater efficacy in treating CIA by treatment with the dAbm16/IL-1ra fusion rather than IL1-ra alone at the same mg/kg dose is despite the fact that the fusion provides half the moles of IL-1ra compared to IL-1ra alone.

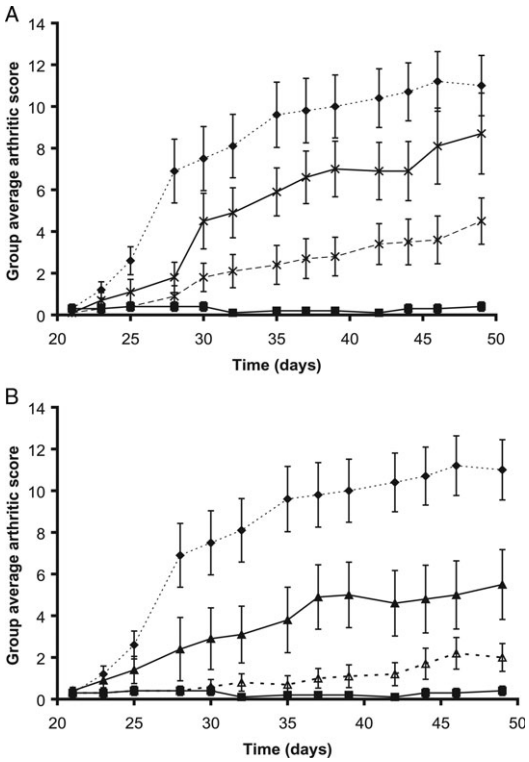

Fig. 4. *In vivo* efficacy of an AlbudAb fusion in a collagen induced arthritis (CIA) model. Comparison of the effect of (**A**) 1 mg/kg and (**B**) 10 mg/kg dosing of AlbudAb/IL-1ra fusion with the effect of dosing IL-1ra alone on the development of CIA in mice. Groups of 10 mice were injected with collagen at day 0 and from 21 to 49 days were treated three times weekly with - - ◆ - - saline, —✕— 1 mg/kg IL-1ra, —▲— 10 mg/kg IL-1ra, - - ✳ - - 1 mg/kg dAbm16/IL-1ra fusion or - - △ - - 10 mg/kg dAbm16/IL-1ra fusion by intraperitoneal injection or with —■— 0.4 mg/kg positive control steroid, dexamethasone, by subcutaneous injection. Animals were scored at each treatment for severity of arthritis in each limb on a scale of 0 to 4 for increasing severity (see Methods). Individual animals were removed from the study for ethical reasons if the score is greater than 12. Mean scores for each group of 10 animals were calculated and recorded ± SEM on the chart (using a statistically conservative score of 16 for any animals removed from the study).

Discussion

A general advantage of the AlbudAb approach compared to other technologies for extending the serum half-lives of proteins, such as albumin (Syed *et al*., 1997; Osborn *et al*., 2002) and Fc fusions (Mohler *et al*., 1993) is the small size of the dAb fusion partner. At ~12 kDa, an AlbudAb provides only a small increase in molecular weight to a protein drug, meaning that for a given mg/kg dose, an AlbudAb conjugate will provide more moles of the therapeutic moiety. This is especially important if the therapeutic entity is a small protein or a peptide, where the molecule that confers

the extended half-life may constitute the vast majority of the protein in the vial. Furthermore, in contrast to Fc fusions, AlbudAb fusions can be expressed at high yields in bacterial culture. Thus, we are able to take advantage of the long serum half-life conferred on SA and Fc fusions by recycling via the FcRn receptor (Chaudhury *et al*., 2003) without the size and manufacturing disadvantages.

It is interesting to note that even though the size of an AlbudAb (~12 kDa) is well below the glomerular filtration threshold of 60 kDa, the serum half-life of dAbm16 in a mouse (24 h) is very similar to the half-life previously recorded for MSA (35 h in mouse) (Chaudhury *et al*., 2003) and that the serum half-lives of dAbr3 and dAbr16 in a rat (53 and 43 h, respectively) are very similar to that of RSA (53 hours in this study). Given that the half-life of SA in humans is in the region of 19 days (Peters, 1985), we would therefore expect AlbudAbs that bind human albumin to have terminal half-lives in man of approximately 19 days and AlbudAb-drug fusions to have half-lives of 19 days unless some active mechanism mediated by the therapeutic moiety degrades or otherwise leads to the clearance/inactivation of the fusion. However, even in the case of our AlbudAb-IL-1ra fusion, which appears to be subject to some form of IL-1ra specific clearance, the dramatic improvement in *in vivo* half-life and efficacy we observed in mice would allow daily dosing in man to be replaced by weekly dosing, whilst also improving the therapeutic effect.

Here we have shown that a drug's half-life and efficacy can be dramatically improved by direct fusion to an albumin binding antibody fragment. The conjugation of IL-1ra to an AlbudAb has a significant effect on its *in vivo* potency, purely by improving its pharmacokinetics. The improvement in PK profile is sufficient to improve efficacy despite a lower molar dose of IL-1ra. We believe that our approach is directly applicable to many other drugs especially those that act without the need for cellular internalization. This includes not only proteins such as interferon alpha and GM-CSF, but also peptides such as a peptide antagonist of TNFα (Chirinos-Rojas *et al*., 1998) and small molecule drugs such as methotrexate (Rau and Herborn, 2004). In comparison with other technologies for extending serum half-life including Fc or albumin fusion and PEGylation, the AlbudAb fusion technology has several advantages. It avoids the reliance on mammalian cell culture required for Fc fusions, the need for chemical conjugation and re-purification required for PEGylated drugs and provides significant advantages in terms of the number of moles of drug per gram compared to all three approaches. In summary, AlbudAbs, with their fully human scaffolds, provide a new way to access efficacy improvements by improving serum half-life.

AlbudAb, AlbudAbs, Domain Antibody, Domain Antibodies, dAb and dAbs are trademarks of the GlaxoSmithKline group of companies.

Funding

The authors were all employees of Domantis Ltd at the time this work was carried out.

References

Akeson,A.L *et al*. (1996) *J. Biol. Chem.*, **271**, 30517–30523.
Brand,D.D., Kang,A.H. and Rosloniec,E.F. (2004) *Methods Mol. Med.*, **102**, 295–312.
Chapman,A.P. (2002) *Adv. Drug Deliv. Rev.*, **54**, 531–545.
Chaudhury,C., Mehnaz,S., Robinson,J.M., Hayton,W.L., Pearl,D.K., Roopenian,D.C. and Anderson,C.L. (2003) *J. Exp. Med.*, **197**, 315–322.
Chirinos-Rojas,C.L., Steward,M.W. and Partidos,C.D. (1998) *J. Immunol.*, **161**, 5621–5626.
Choy,E.H., Hazleman,B., Smith,M., Moss,K., Lisi,L., Scott,D.G., Patel,J., Sopwith,M. and Isenberg,D.A. (2002) *Rheumatology (Oxford)*, **41**, 1133–1137.
Courtenay,J.S., Dallman,M.J., Dayan,A.D., Martin,A. and Mosedale,B. (1980) *Nature*, **283**, 666–668.
Dennis,M.S., Zhang,M., Meng,Y.G., Kadkhodayan,M., Kirchhofer,D., Combs,D. and Damico,L.A. (2002) *J. Biol. Chem.*, **277**, 35035–35043.
Halpern,W. *et al*. (2002) *Pharm. Res.*, **19**, 1720–1729.
Jain,R.K. (1988) *Cancer Res.*, **48**, 2641–2658.
Jespers,L., Schon,O., Famm,K. and Winter,G. (2004a) *Nat. Biotechnol.*, **22**, 1161–1165.
Jespers,L., Schon,O., James,L.C., Veprintsev,D. and Winter,G. (2004b) *J. Mol. Biol.*, **337**, 893–903.
Jiang,Y., Genant,H.K., Watt,I., Cobby,M., Bresnihan,B., Aitchison,R. and McCabe,D. (2000) *Arthritis Rheum.*, **43**, 1001–1009.
Holliger,P., Wing,M., Pound,J.D., Bohlen,H. and Winter,G. (1997) *Nat. Biotechnol.*, **15**, 632–636.
Lee,H., Kimko,H.C., Rogge,M., Wang,D., Nestorov,I. and Peck,C.C. (2003) *Clin. Pharmacol. Ther.*, **73**, 348–365.
Mainardes,R.M. and Silva,L.P. (2004) *Curr. Drug Targets*, **5**, 449–455.
McCafferty,J., Griffiths,A.D., Winter,G. and Chiswell,D.J. (1990) *Nature*, **348**, 552–554.
Mohler,K.M., Torrance,D.S., Smith,C.A., Goodwin,R.G., Stremler,K.E., Fung,V.P., Madani,H. and Widmer,M.B. (1993) *J. Immunol.*, **151**, 1548–1561.
Nguyen,A., Reyes,A.E., 2nd, Zhang,M., McDonald,P., Wong,W.L., Damico,L.A. and Dennis,M.S. (2006) *Protein Eng. Des. Sel.*, **19**, 291–297.
Osborn,B.L., Olsen,H.S., Nardelli,B., Murray,J.H., Zhou,J.X., Garcia,A., Moody,G., Zaritskaya,L.S. and Sung,C. (2002) *J. Pharmacol. Exp. Ther.*, **303**, 540–548.
Peters,T., Jr (1985) *Adv. Protein Chem.*, **37**, 161–245.
Pockros,P.J. *et al*. (2004) *Am. J. Gastroenterol.*, **99**, 1298–1305.
Rau,R. and Herborn,G. (2004) *Clin. Exp. Rheumatol.*, **22**, S83–S94.
Sarkar,C.A., Lowenhaupt,K., Horan,T., Boone,T.C., Tidor,B. and Lauffenburger,D.A. (2002) *Nat. Biotechnol.*, **20**, 908–913.
Shuck,M.E., Eessalu,T.E., Tracey,D.E. and Bienkowski,M.J. (1991) *Eur. J. Immunol.*, **21**, 2775–2780.
Sjobring,U., Falkenberg,C., Nielsen,E., Akerstrom,B. and Bjorck,L. (1988) *J. Immunol.*, **140**, 1595–1599.
Smith,B.J. *et al*. (2001) *Bioconjug. Chem.*, **12**, 750–756.
Syed,S., Schuyler,P.D., Kulczycky,M. and Sheffield,W.P. (1997) *Blood*, **89**, 3243–3252.
Veronese,F.M. and Pasut,G. (2005) *Drug Discov. Today.*, **10**, 1451–1458.
Ward,E.S., Gussow,D., Griffiths,A.D., Jones,P.T. and Winter,G. (1989) *Nature*, **341**, 544–546.
Werle,M. and Bernkop-Schnürch,A. (2006) *Amino Acids*, **30**, 352–367.
Winter,G., Griffiths,A.D., Hawkins,R.E. and Hoogenboom,H.R. (1994) *Annu. Rev. Immunol.*, **12**, 433–455.
Wunder,A. *et al*. (2003) *J. Immunol.*, **170**, 4793–4801.

Received October 22, 2007; revised October 22, 2007; accepted October 24, 2007

Edited by Peter Hudson