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CD14 is a ligand for the integrin α4β1

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Abstract **Cell adhesion mediated by the integrin α4β1 plays a key role in many biological processes reflecting both the number and functional significance of α4β1 ligands. The lipopolysaccharide (LPS) receptor, CD14, is a GPI-linked cell surface glycoprotein with a wide range of reported functions and associations, some of which overlap with that of α4β1. This overlap led us to test the specific hypothesis that α4β1 and CD14 interact directly. Jurkat T cells (α4β1$^+$) were found to adhere to a recombinant CD14-Fc protein via α4β1, whilst K562 cells (α4β1$^-$) did not. However, stable reexpression of the α4-subunit conferred this ability. The adhesion of both cell types to CD14 displayed activation state-dependent binding very similar to the interaction of α4β1 with its prototypic ligand, VCAM-1. In solid-phase assays, CD14-Fc bound to affinity-purified α4β1 in a dose-dependent manner that was induced by activating anti-β1 mAbs. Finally, in related experiments, JY cells (α4β7$^+$) were also found to attach to CD14-Fc in an α4-dependent manner. In summary, CD14 is a novel ligand for α4β1, exhibiting similar activation-state dependent binding characteristics as other α4β1 ligands. The biological relevance of this interaction will be the subject of further studies.**

Keywords: Integrin; CD14; Cell adhesion

1. Introduction

Integrins are α,β heterodimeric cell surface adhesion receptors that mediate cell–cell and cell–matrix interactions [1]. A major functional role of integrins is environmental sensing, which they achieve through the spatiotemporal control of cell signalling at cell adhesion contacts. Current models of integrin function envisage conformational changes occurring between and/or within the integrin subunits that lead to altered receptor activation states. Conformational changes induced by extracellular ligand binding or cytoplasmic factors are known as 'outside-in' and 'inside-out' activation, respectively [2,3]. The bidirectional responsiveness of integrins therefore allows them to regulate the strengthening of cell–matrix interactions and to initiate new cell adhesion contacts [4].

Integrins bind to all of their characterised ligands via a critical acidic motif in a divalent cation-dependent manner [5]. The α4β1 integrin (also known as VLA-4 or CD49d/CD29) is expressed on the surface of a variety of cell types, including lymphocytes, monocytes, eosinophils and basophils [6], and has so far been reported to bind to a wide variety of cell surface and extracellular matrix ligands [7] making it one of the more promiscuous integrins. These binding partners include the classical α4β1 ligands, vascular cell adhesion molecule (VCAM-1) [8] and the alternatively spliced type III connecting segment of fibronectin (FN) [9,10], together with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [11], osteopontin [12], and thrombospondin [13]. Through these interactions, α4β1 plays an important role in embryonic development, adult homeostasis, and immune responses [6,14].

CD14 is a 55 kDa GPI-anchored, leucine-rich repeat (LRR) protein [15–17]. CD14 is expressed strongly on the surface of monocytes and other myeloid cells, and is expressed at lower levels on B-lymphocytes, basophils, mammary cells, placental trophoblasts, gingival fibroblasts [18] and low passage number endothelial cells [19,20]. A soluble form of CD14 is also present in serum at μg/ml levels. CD14 is the major receptor for bacterial endotoxin (lipopolysaccharide, LPS) and mediates innate immune responses [21,22]. The interaction of LPS with CD14 results in the release of inflammatory cytokines and the upregulation of adhesion molecules (β1 integrin and αLβ2) [21]. As CD14 does not possess a transmembrane domain, it is envisaged that signals arising from the LPS/CD14 interaction require a signalling partner to elicit a response. The Toll-like receptors, CD55 and integrins have all been suggested to fulfil this role indirectly through the formation of activation clusters [23]. CD14 is known to have other functions, such as the phagocytosis of apoptotic cells [24], and this has also been postulated to be mediated via an integrin signalling partner [25]. In support of the role of integrins in such processes, CD14 has been reported to be in close proximity to αMβ2 by resonance energy transfer techniques, although in an LPS-dependent manner [26]. In addition, studies have shown that an unidentified 50 kDa protein on the surface of endothelial cells can support α4-mediated cell adhesion [27], and CD14 on endothelial cells is required for LPS-induced rolling, adhesion and transmigration of leukocytes [20]: processes that can be mediated by α4 integrins [6]. Therefore, the overlapping functions of α4β1 and CD14 in immune responses led us to investigate the possibility that they might interact directly. Using a variety of cell-based and purified protein binding assays, we find that CD14 can serve as a ligand for α4β1.

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Abbreviations: mAb, monoclonal antibody; VCAM-1, vascular cell adhesion molecule; MAdCAM-1, mucosal addressin cell adhesion molecule-1; FN, fibronectin; $FNIII_{(6–10)}$, recombinant fragment of FN comprising type III repeats 6–10; HBS, HEPES-buffered saline; LPS, lipopolysaccharide; TPA, phorbol 12-myristate 13-acetate

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2. Materials and methods

2.1. General reagents and cell culture

Monoclonal antibodies (mAb) used were: 12G10, mouse anti-human integrin β1 [28]; mAb13, rat anti-human integrin β1 (a gift from K. Yamada, NIH, Bethesda, MD, USA); TS2/16, mouse anti-human integrin β1 (a gift from A. Sonnenberg, Netherlands Cancer Institute, Amsterdam, The Netherlands); K20, mouse anti-human integrin β1 (Beckman Coulter, High Wycombe, UK); HP2/1 and 44H6, mouse anti-human integrin α4 (Serotec, Oxford, UK); 8F2, mouse anti-human integrin α4 (a gift from C. Morimoto, DFCI, Boston, MA, USA); and JBS5, mouse anti-human integrin α5 (Serotec); A polyclonal sheep anti-human CD14 antibody (AB383) was purchased from R& D Systems (Abingdon, UK).

The recombinant fragment of fibronectin (FN) comprising type III repeats 6–10 [$FNIII_{(6-10)}$] and containing the recognition sequence for α5β1 was purified as previously described [29], as was the control α5β1-Fc fusion protein [30].

All cell lines were from the European Collection of Animal Cell Cultures (ECACC) unless otherwise stated, passaged every 3–4 days and cultured at 37 °C and 5% CO_2 in defined medium as follows. COS-1 African green monkey kidney cells were cultured in Dulbecco's minimal essential medium (DMEM) with 0.11 g/l sodium pyruvate and pyridoxine supplemented with 10% (v/v) foetal calf serum (FCS), 1% (v/v) L-glutamine. Cells were detached from flasks by treatment with 1× trypsin/EDTA for 5 min for routine passaging. The MOLT-4 human lymphoblastic leukaemia suspension cell line, the K562 human chronic myelogenous leukaemia suspension cell line, the Jurkat human T cell lymphoblastic leukaemia suspension cell line (a gift from P. Shore, University of Manchester), and the JY human cell line were cultured in RPMI 1640, 10% (v/v) FCS, 1% (v/v) L-glutamine. An addition of 1 mg/ml G418 (GIBCO BRL) was made for cells transfected with the appropriate expression vector.

2.2. Transfection of K562 cells with α4 integrin

Transfection and enrichment of K562 cells expressing the α4 integrin subunit was as previously described [29].

2.3. Production of soluble Fc-fusion proteins

Recombinant soluble human CAM-Fc fusion proteins were produced in COS-1 cells as previously described [11]. VCAM-1-Fc comprises the Ig domains 1 and 2. CD14-Fc was a gift from D. Simmons (GlaxoSmithKline, Uxbridge, UK), the protein comprising residues T1-R335 of the mature protein [16].

2.4. Cell attachment assays

Cell attachment assays were performed as previously described [29]. Briefly, 96-well plates (Costar Corning) were coated for 60–90 min with protein ligands diluted in phosphate-buffered saline (PBS, GIBCO BRL) before blocking with heat-denatured bovine serum albumin (BSA). K562 or Jurkat cells were washed, resuspended at 1–1.5×10^6 cells/ml, and 50 μl aliquots added to wells containing HEPES-buffered saline (HBS) containing 2× final concentration of antibodies and/or cations for 30 min at 37 °C and 5% (v/v) CO_2. Unbound cells were removed by aspiration and gentle washing. Bound cells were fixed by addition of 5% (v/v) glutaraldehyde in HBS, stained with 0.1% (w/v) crystal violet in 200 mM methylethanesulfonic acid (MES) pH 6 and dye eluted with 10% (v/v) acetic acid. To assess the total number of cells added, 100%, 75%, 50%, 25% and 0% cells were added to wells and fixed by the addition of 1/10 volume of 50% (v/v) glutaraldehyde and stained as above. Absorbance (570 nm) of each well was measured with a multiscan plate reader.

2.5. Affinity-purification of α4β1

Purification of α4β1, using mAb13, from MOLT-4 cells was as previously described [29].

2.6. Solid-phase receptor-ligand binding assay

Assays were performed as previously described [29]. Briefly, Costar high-binding microtitre plates (Corning Costar) were coated with α4β1 integrin (diluted 1:100 in PBS) overnight. Wells were blocked for 3 h with 5% (w/v) BSA, 150 mM NaCl, 0.05% (w/v) NaN_3, 25 mM Tris–HCl, pH 7.4 and washed with TBS plus 1 mM $MnCl_2$ and 0.1% (w/v) BSA (TBS-Mn). Anti-integrin antibodies at 20 μg/ml were added to wells in TBS-Mn. To study the effects of other divalent cations, $MgCl_2$ or $CaCl_2$ replaced $MnCl_2$ in this buffer. A range of CD14-Fc concentrations in TBS-Mn was also added to wells and plates incubated at 37 °C for 3 h. After washing (TBS-Mn), bound CD14-Fc was detected with horseradish peroxidase-conjugated anti-human-Fc antibody (Sigma) and ABTS substrate with absorbance readings at 405 nm measured using a multiscan plate reader. Background attachment to BSA-blocked wells was subtracted from all measurements.

3. Results

3.1. Cells attach to CD14 via the integrin α4β1

To ascertain whether cells expressing α4β1 could adhere to CD14, Jurkat cells were incubated with CD14-Fc-coated 96-well plates. Cells adhered in a dose-dependent manner in the presence of 1 mM Mn^{2+}, with maximal adhesion observed at a concentration of 10 μg/ml of CD14-Fc (Fig. 1A). The anti-functional anti-α4 (HP2/1) and anti-β1 (mAb13) mAbs inhibited Jurkat cell attachment compared to the non-functional anti-α4 (44H6) and anti-β1 (K20) mAbs. Thus, attachment to CD14-Fc was demonstrated to be mediated by α4β1 (Fig. 1B).

The K562 cell line does not express the α4-subunit, but does express α5β1. K562 cells therefore adhere to α5β1 ligands, such as a 50 kDa fragment of FN, but do not adhere to α4β1 ligands, such as VCAM-1 (Fig. 2A). Consistent with the data from Jurkat cell adhesion studies, K562 cells did not attach to CD14-Fc (Fig. 2A). We therefore tested whether expression of α4 on K562 cells could confer the ability to interact with CD14-Fc via α4β1. K562 cells expressing α4 (K562-α4) attached to both VCAM-1-Fc and CD14-Fc in an α4β1-dependent manner, in the presence of 1 mM Mn^{2+}, as demonstrated by addition of anti-functional mAbs directed against the α4 subunit (Fig. 2B). K562-α4 cells also attached to CD14-Fc in a dose-dependent manner (Fig. 2C). Both K562-α4 and Jurkat cells did not adhere to a control Fc-fusion protein comprising the extracellular domains of α5β1 (less than 5% of the added cells attached at 10 μg/ml; data not shown).

3.2. Cell attachment to CD14 via α4β1 is activation state-dependent

Integrin-mediated adhesive events can be modulated by mAbs and/or divalent cations. We therefore investigated the activation state-dependence of α4β1-mediated adhesion to CD14-Fc. Jurkat cell attachment performed in HBS only, or in HBS supplemented with 1 mM Mg^{2+} and 1 mM Ca^{2+}, resulted in basal levels of cell attachment to CD14-Fc (Fig. 3A). Cell attachment assays performed in 1 mM Mn^{2+}, however, resulted in augmented cell attachment. Identical results were obtained for the α4β1 ligand VCAM-1-Fc (data not shown). This is consistent with the previously characterised effects of these cations on integrin function.

The anti-β1 mAbs 12G10 and TS2/16 are well-characterised activating anti-integrin mAbs. Cell attachment assays performed in HBS+Mg^{2+}/Ca^{2+} in the presence of either 12G10 or TS2/16 demonstrated increased levels of cell attachment to CD14-Fc, comparable to that induced by Mn^{2+} (Fig. 3A). This effect was not seen in the presence of the non-functional anti-β1 mAb, K20. The equivalent experiments performed in

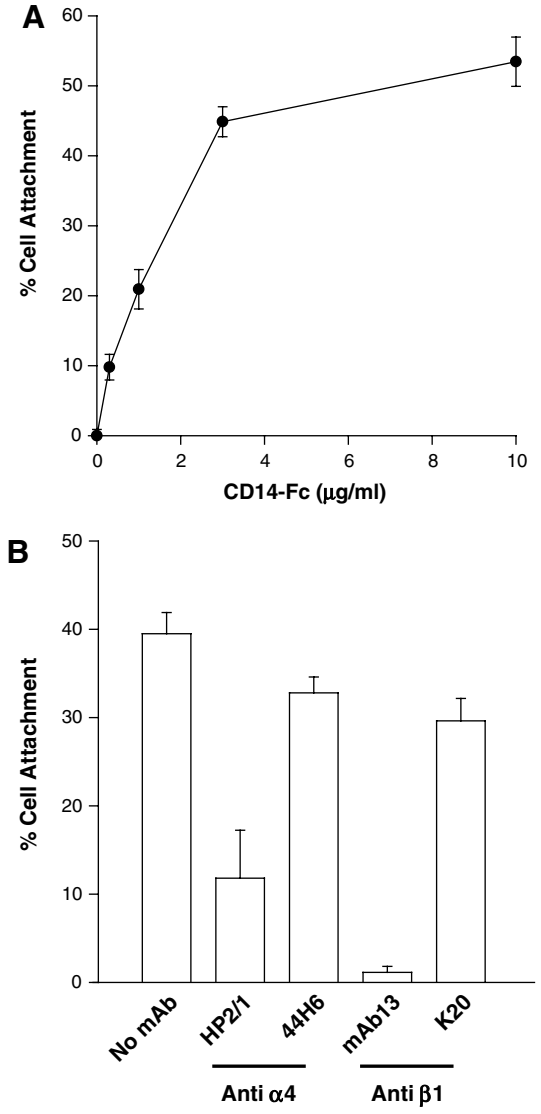

Fig. 1. Jurkat cells attach to CD14-Fc via α4β1. Jurkat cells expressing endogenous α4β1 were allowed to attach to (A) a range of concentrations of CD14-Fc or (B) 3 μg/ml CD14-Fc in the presence of HP2/1 (anti-functional anti-α4 mAb), 44H6 (non-functional anti-α4 mAb), mAb13 (anti-functional anti-β1 mAb) or K20 (non-functional anti-β1 mAb). Assays were performed in buffers containing 1 mM Mn^{2+}. Data points represent means ± S.D.

HBS + Mn^{2+} did not produce such dramatic increases in cell attachment, reflecting the already increased activation state of the integrin due to the presence of Mn^{2+}. Identical results were obtained for the α4β1 ligand VCAM-1-Fc (data not shown).

Phorbol esters, such as phorbol 12-myristate 13-acetate (TPA), have been demonstrated to enhance integrin-mediated adhesive events through stimulating clustering or receptor delivery, i.e. by a mechanism that does not involve the modulation of the affinity of the integrin for its ligand. We therefore investigated whether α4β1-mediated cell adhesion to CD14-Fc could be modulated by TPA. Jurkat cell attachment to CD14-Fc in the presence of TPA was significantly enhanced. The anti-functional mAb HP2/1 completely inhibited cell adhesion under these conditions unlike the non-functional mAb 44H6,

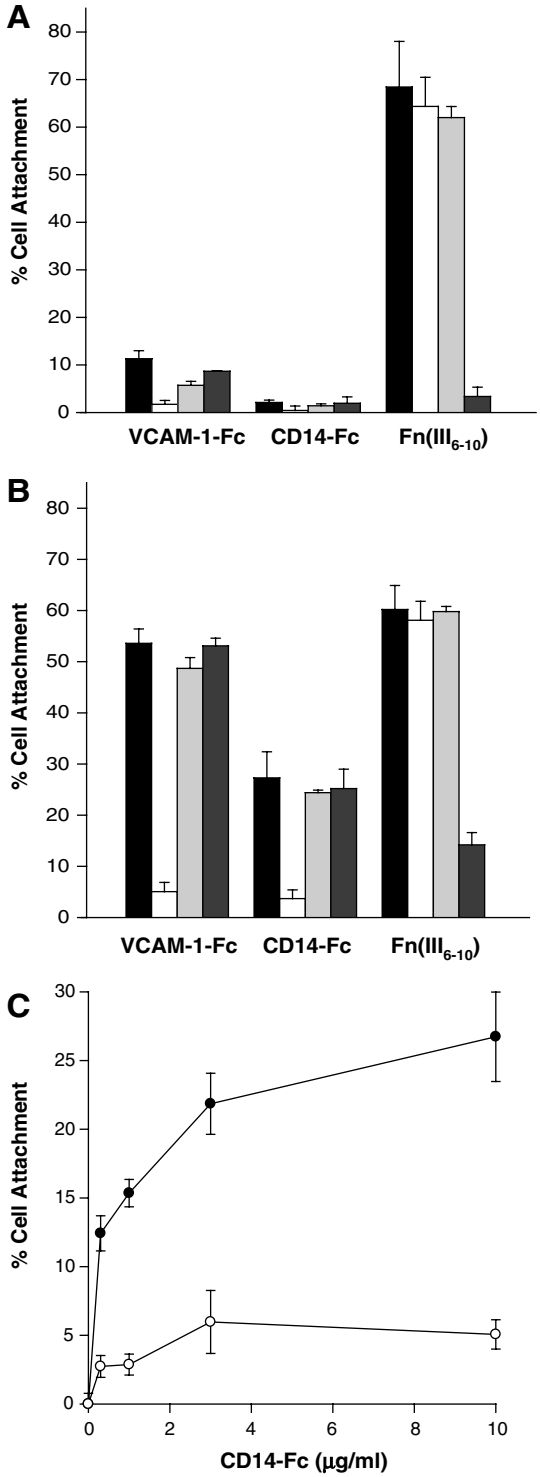

Fig. 2. K562-α4 cells attach to CD14-Fc via α4β1. (A) Untransfected K562 cells and (B) K562-α4 cells were allowed to attach to 3 μg/ml VCAM-1-Fc, CD14-Fc or $FNIII_{(6-10)}$ only (black bars), or in the presence of HP2/1 (anti-functional anti-α4 mAb; white bars), 44H6 (non-functional anti-α4 mAb; light grey bars) or JBS5 (anti-functional anti-α5 mAb; dark grey bars). (C) Untransfected K562 cells (open circles) and K562-α4 cells (filled circles) were allowed to attach to a range of concentrations of CD14-Fc. Assays were performed in buffers containing 1 mM Mn^{2+}. Data points represent means ± S.D.

demonstrating that the cell attachment was mediated by α4β1 (Fig. 3B).

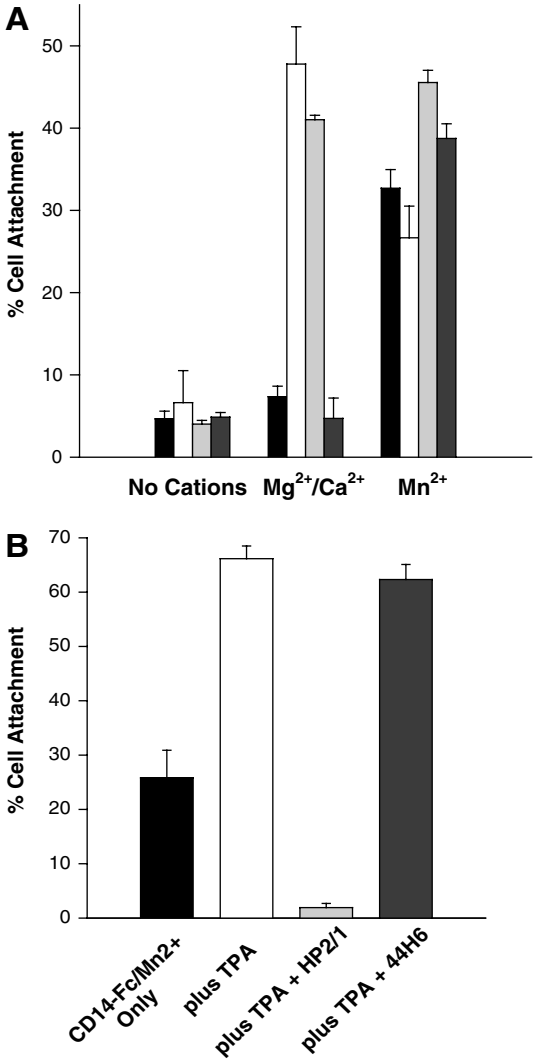

Fig. 3. Activation-state dependence of cell attachment to CD14-Fc. (A) Jurkat cells were allowed to attach to 10 μg/ml CD14-Fc alone (black bars), or in the presence of 12G10 (activating anti-β1 mAb; white bars), TS2-16 (activating anti-β1 mAb; light grey bars) or K20 (non-functional anti-β1 mAb; dark grey bars). Divalent cation conditions (1 mM each) are indicated below the *x*-axis. (B) Jurkat cells were allowed to attach to CD14-Fc (10 μg/ml) in DMEM/HEPES plus 0.5 mM Mn^{2+} alone (black bar), or in the presence of TPA at 50 ng/ml (white bar), or TPA plus HP2/1 (anti-functional anti-α4 mAb; light grey bar), or TPA plus 44H6 (non-functional anti-α4 mAb; dark grey bar). Data points represent means ± S.D.

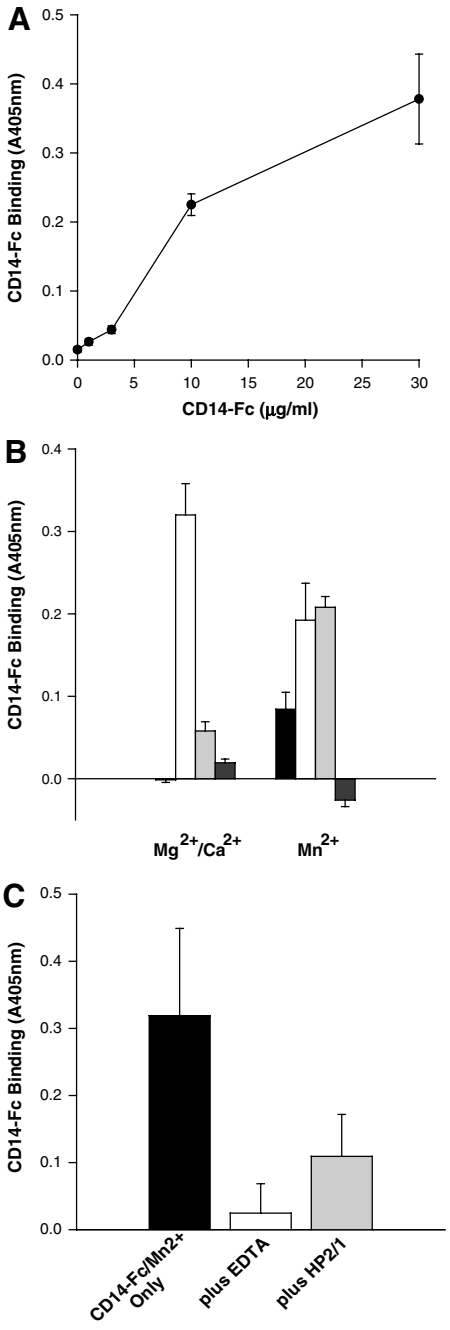

Fig. 4. Affinity purified α4β1 binds to CD14-Fc in an activation state-dependent manner. (A) Dose dependency of soluble CD14-Fc binding to affinity-purified α4β1 immobilised on a 96-well plate. (B) Soluble CD14-Fc at 10 μg/ml was allowed to bind to immobilised α4β1 alone (black bars), or in the presence of 12G10 (activating anti-β1 mAb; white bars), TS2-16 (activating anti-β1 mAb; light grey bars) or 5 mM EDTA (divalent cation chelator; dark grey bars). Divalent cation conditions are indicated below the *x*-axis (2 mM each). (C) Affinity purified α4β1 was allowed to bind to immobilised CD14-Fc plus 2 mM Mn^{2+} alone (black bar), or in the additional presence of 5 mM EDTA (divalent cation chelator; white bar) or HP2/1 (anti-functional anti-α4 mAb; grey bar). Bound α4β1 was detected by the non-functional anti-α4 mAb 44H6. Data points represent means ± S.D.

3.3. CD14-Fc interacts with affinity-purified α4β1

To test for a direct α4β1/CD14-Fc interaction, the ability of affinity-purified α4β1 from MOLT-4 cells to bind to CD14-Fc was assessed in a solid phase assay format. CD14-Fc bound to affinity-purified α4β1, coated onto the surface of 96-well plates, in a dose-dependent manner in the presence of Mn^{2+} (Fig. 4A). This interaction was inhibited by chelation of divalent cations with EDTA, and augmented by the activating anti-β1 mAbs 12G10 and TS2/16 (Fig. 4B). Marked stimulation of CD14-Fc binding to purified α4β1 by 12G10, was also observed in 1 mM Mg^{2+}/Ca^{2+} (Fig. 4B). The CD14-Fc/α4β1 interaction was also observed in an inverted assay format, i.e. CD14-Fc being coated on the plate and subsequent incubation with α4β1 in solution. Binding of α4β1 to CD14-Fc was again inhibited by EDTA and HP2/1 (Fig. 4C). The α4β1 ligand VCAM-1-Fc behaved in a similar manner to CD14-Fc in all of these assays (data not shown). Taken together, these

data demonstrate that the purified CD14-Fc and α4β1 proteins can interact directly in a manner that resembles other α4β1 ligands.

3.4. LPS does not modulate Jurkat cell attachment to CD14-Fc

LPS is a well-characterised ligand for CD14. We therefore sought to investigate whether LPS binding to CD14-Fc modulated the ability of Jurkat cells to attach to CD14-Fc via α4β1. Cell attachment assays performed in the presence of a range of concentrations of LPS did not alter Jurkat cell attachment to CD14-Fc (Fig. 5). Furthermore, an antibody that specifically inhibits the interaction of CD14 with LPS (as measured by the ability to inhibit LPS-induced TNF-α release from cells) had no effect on either Jurkat or K562-α4 cell attachment to CD14-Fc or VCAM-1-Fc (data not shown). The polyclonal anti-CD14 antibody, however, did specifically recognise the recombinant CD14-Fc protein immobilised to plastic in an ELISA type assay (data not shown). Taken together, these findings indicate that LPS does not modulate cell attachment to CD14, and suggest that the region of CD14-Fc responsible for the interaction with LPS and α4β1 are not the same.

3.5. CD14-Fc is also a ligand for α4β7

The α4β1 and α4β7 integrins are able to bind to common ligands such as VCAM-1 and MAdCAM-1. We therefore investigated whether α4β7 could use CD14 as a ligand in cell attachment assays. The $\alpha4\beta7^{+}/\alpha4\beta1^{-}$ JY cell line attached to CD14-Fc in a dose-dependent manner with approximately 50% of added cells being adherent to 10 μg/ml of coated CD14-Fc (data not shown). The anti-functional anti-β1 mAb, mAb13, did not inhibit cell attachment, consistent with these cells not expressing the β1-subunit. The anti-functional anti-α4 mAb, HP2/1, virtually ablated JY cell attachment to CD14-Fc, in contrast to the minor effects of the non-functional anti-α4 mAbs, 44H6 and 8F2 (Fig. 6).

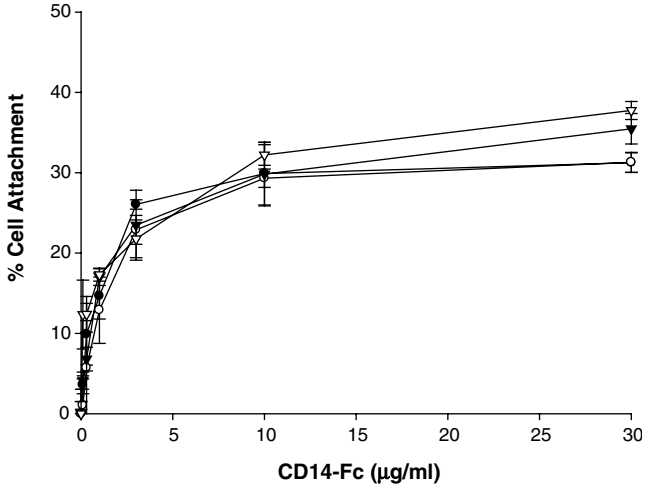

Fig. 5. LPS modulation of Jurkat cell attachment to CD14-Fc. Jurkat cells were allowed to attach to a range of concentrations CD14-Fc in the presence of 1 mM Mn^{2+}, 0.25% normal human serum and a range of concentrations of LPS (0 μg/ml closed circles; 0.2 μg/ml open circles; 2 μg/ml closed triangles; and 20 μg/ml open triangles) from *E. coli* 0111:B4. Data points represent means ± S.D.

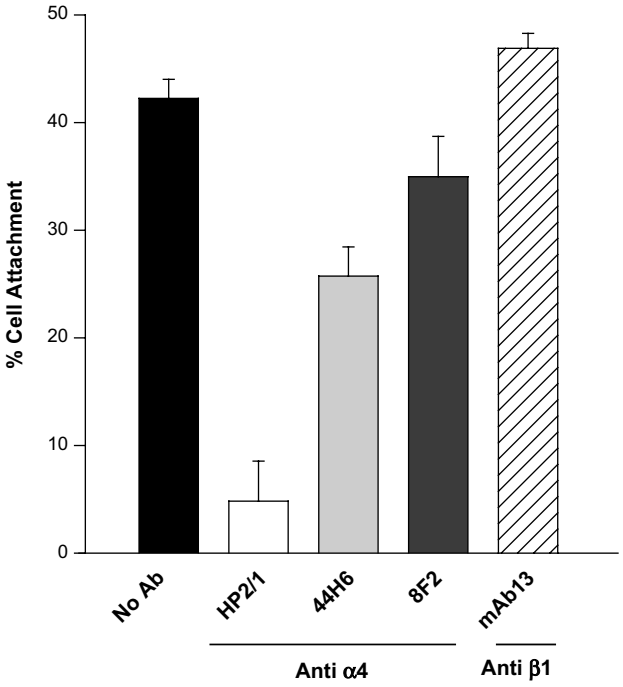

Fig. 6. JY cells attach to CD14-Fc. JY cells expressing endogenous α4β7 were allowed to attach to 10 μg/ml CD14-Fc only (black bar), or in the presence of HP2/1 (anti-functional anti-α4 mAb; white bar), 44H6 (non-functional anti-α4 mAb; light grey bar), 8F2 (non-functional anti-α4 mAb; dark grey bar) or mAb13 (anti-functional anti-β1 mAb; hatched bar). Data points represent means ± S.D.

4. Discussion

The main findings from these studies are that CD14 is a novel ligand for α4β1, and that this interaction has similar activation-state dependent binding characteristics as other α4β1 ligands, such as VCAM-1. The CD14-α4β1 interaction was observed in cell attachment assays with Jurkat cells that express endogenous α4β1 and in K562 cells transfected with the α4-subunit, but not in untransfected K562 cells. The direct nature of this interaction was verified by protein–protein solid phase assays and together with the ability of anti-functional anti-α4 mAbs to perturb the adhesion, these findings demonstrate that the interaction of the cells with CD14 is via α4β1. It may be speculated that the integrins α4β7 and α9β1, which interact with many of the same ligands as α4β1, could also bind CD14. In support of this hypothesis, we have presented data for the α4β7-expressing JY cell line, indicating that cell surface α4β7 can also serve as a receptor for CD14.

CD14 is a receptor for bacterial lipopolysaccharide (LPS) and as such is involved in the innate immune response. Recognition of LPS by the innate immune system elicits pro-inflammatory responses, e.g. the release of cytokines and upregulation of adhesion molecules such as β1-integrins and αLβ2, which can eventually cause fatal sepsis syndrome in humans [21,22]. As CD14 does not possess a transmembrane domain, it is not capable of initiating a classical transmembrane signal. Indirect interactions of CD14 with integrins or Toll-like receptors (TLRs) have therefore been suggested to fulfil these signalling functions via complex cytoplasmic signalling pathways that include the phosphorylation of focal

adhesion-associated proteins such as paxillin and Pyk2 [31]. Recently, the recruitment of CD14 into signalling complexes within lipid raft domains has been suggested as an alternative signalling mechanism [23,32]. An interesting corollary of these data was the identification of the α4β1-binding partner CD81 in lipid rafts, and lipid rafts have been shown to regulate the function of α4β1 [33]. Other functions for CD14 have been demonstrated, including LPS-mediated bone resorption, the regulation of apoptosis and internalisation of apoptotic bodies, and an involvement in leukocyte–endothelial cell interactions [20,21,34]. The α4β1/CD14 interaction defined here may play a role in any of the above processes, and further work will be required to assess this possibility.

Key questions still remain regarding the mechanism of binding of CD14 to α4β1 and the physiological consequences of this interaction. The LRR has been implicated as a protein recognition motif, which typically adopts a horseshoe-like structure. Residues comprising the concave face and adjacent loops of the structure are most commonly implicated as the protein interaction surfaces [35]. There are precedents for the interaction of integrins with LRR proteins, e.g. decorin/α2β1 [36] and CD14/αMβ2 [37]. Also the structure of the LRR-containing platelet glycoprotein Ibα N-terminal domain has been solved that is known to interact with the A1 domain of von Willebrand factor [38]. A-domains are also found in integrins and are involved in ligand binding, suggesting a link between integrin-ligand binding and LRR-containing proteins. An acidic residue is required for the cation-dependent interaction of integrins with their ligands, and the CD14 protein contains many (>15) conserved acidic residues between human and mouse [15]. Some of these residues are found within the LRR repeats and may be structurally important. Future site-directed mutagenesis experiments targeting these residues may aid in the identification of key residues involved with the interaction with α4β1. The binding site for CD14 with LPS has been identified in the N-terminal region of the CD14 protein [21]. This site does not appear to involve the same residues as the site for interaction with α4β1, as a polyclonal antibody directed against CD14 that inhibits LPS-induced TNF-α release did not inhibit the interaction of α4β1-expressing cells with CD14 and the presence of LPS did not modulate α4β1-mediated cell attachment to CD14-Fc.

Soluble CD14 has been reported to interact with an unknown membrane protein on monocytes resulting in the release of pro-inflammatory cytokines such as TNF-α [18]. A simple hypothesis was therefore that α4β1 is the unknown membrane receptor that interacts with CD14 to produce TNF-α. This is not the case as anti-functional mAbs that disrupt the α4β1–CD14 interaction do not inhibit the CD14-induced release of TNFα from monocytes (data not shown). However, the cell attachment of THP-1 cells to CD14-Fc in these studies was not inhibited by anti-functional α4 mAbs, suggesting that these cells did not use α4β1 to attach to CD14. Jurkat cells that do use α4β1 to adhere to CD14 did not release TNF-α upon incubation with CD14-Fc. As Jurkat is a T cell line it may be pertinent to assess the modulation of CD14-induced release of other cytokines, such as IFN-γ, by α4β1.

The putative role of the interaction of α4β1 with CD14 in the innate response may therefore be more complex. It is not known whether the α4β1–CD14 interaction occurs in *cis*, i.e. on the surface of the same cell, or in *trans*, between different cells. Another simple hypothesis could be that the interaction of CD14 with α4β1 simply tethers CD14 close to the cell membrane thereby increasing the local concentration of CD14/LPS in the vicinity of signalling molecules such as the TLRs. Such a mechanism could involve a role for compartmentalisation of these proteins at the cell surface by lipid rafts. LPS and many other molecules have therefore been the focus of drug discovery programmes to aid the treatment of sepsis [39]. Furthering our understanding of the role that the interaction of α4β1 with CD14 plays may lead to novel strategies to address such disease processes.

Overall, these data demonstrate CD14 is a novel ligand for α4β1, and that this interaction has similar activation-state dependent binding characteristics as other α4β1 ligands, such as VCAM-1. This finding may have implications for the role of α4β1 as a signalling partner for CD14 in the innate immune response, in the phagocytosis of apoptotic cells, or in the regulation of leukocyte–endothelial interactions.

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References

[1] Hynes, R.O. (2002) Integrins: bidirectional, allosteric signalling machines. Cell 110, 673–687.
[2] Humphries, M.J., McEwan, P.A., Barton, S.J., Buckley, P.A., Bella, J. and Mould, A.P. (2003) Integrin structure: heady advances in ligand binding, but activation still makes the knees wobble. Trends Biochem. Sci. 28, 313–320.
[3] Travis, M.A., Humphries, J.D. and Humphries, M.J. (2003) An unraveling tale of how integrins are activated from within. Trends Pharmacol. Sci. 24, 192–197.
[4] Geiger, B., Bershadsky, A., Pankov, R. and Yamada, K.M. (2001) Transmembrane extracellular matrix-cytoskeleton crosstalk. Nat. Rev. 2, 793–805.
[5] Xiong, J.P., Stehle, T., Zhang, R., Joachimiak Frech, M., Goodman, S.L. and Arnaout, M.A. (2002) Crystal structure of the extracellular segment of integrin alpha v beta 3 in complex with an Arg-Gly-Asp ligand. Science 296, 151–155.
[6] Rose, D.M., Han, J. and Ginsberg, M.H. (2002) α4 integrins and the immune response. Immunol. Rev. 186, 118–124.
[7] Humphries, J.D., Byron, A. and Humphries, M.J. (2006) Integrin ligands at a glance. J. Cell Sci. 119, 3901–3903.
[8] Elices, M.J., Osborn, L., Takada, Y., Crouse, C., Luhowskyi, S., Hemler, M.H. and Lobb, R.R. (1990) VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 60, 577–584.
[9] Wayner, E.A., Garcia-Pardo, A., Humphries, M.J., McDonald, J.A. and Carter, W.G. (1989) Identification and characterisation of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J. Cell Biol. 109, 1321–1330.
[10] Mould, A.P., Wheldon, L.A., Komoriya, A., Wayner, E.A., Yamada, K.M. and Humphries, M.J. (1990) Affinity chromatographic isolation of the melanoma adhesion receptor for the IIICS region of fibronectin and its identification as the integrin α4β1. J. Biol. Chem. 265, 4020–4024.
[11] Newham, P., Craig, S.E., Seddon, G.N., Schofield, N.R., Rees, A., Edwards, R.M., Jones, E.Y. and Humphries, M.J. (1997) α4 Integrin binding interfaces on VCAM-1 and MAdCAM-1. Integrin binding footprints identify accessory binding sites that play a role in integrin specificity. J. Biol. Chem. 272, 19429–19440.
[12] Bayless, K.G., Meininger, G.A., Scholtz, J.M. and Davis, G.E. (1998) Osteopontin is a ligand for the alpha4beta1 integrin. J. Cell Sci. 111, 1165–1174.
[13] Li, Z., Calzada, M.J., Sipes, J.M., Cashel, J.A., Krutzsch, H.C., Annis, D.S., Mosher, D.F. and Roberts, D.D. (2002) Interactions

of thrombospondins with alpha4beta1 integrin and CD47 differentially modulate T cell behavior. J. Cell Biol. 157, 509–519.
[14] Sheppard, D. (2000) In vivo functions of integrins: lessons from null mutations in mice. Matrix Biol. 19, 203–209.
[15] Ferrero, E., Hsieh, C-L., Francke, U. and Goyert, S.M. (1990) CD14 is a member of the family of leucine-rich proteins and is encoded by a gene syntenic with multiple receptor genes. J. Immunol. 145, 331–336.
[16] Simmons, D.L., Tan, S., Tenen, D.G., Nicholson-Weller, A. and Seed, B. (1989) Monocyte antigen CD14 is a phospholipid anchored membrane protein. Blood 73, 284–289.
[17] Kim, J-I., Lee, C.J., Jin, M.S., Lee, C-H., Paik, S-G., Lee, H. and Lee, J-O. (2005) Crystal structure of CD14 and its implications for lipopolysaccaride signalling. J. Immunol. 280, 11347–11351.
[18] Landmann, R., Link, S., Sansano, S., Rajacic, Z. and Zimmerli, W. (1998) Soluble CD14 activates monocytic cells independently of lipopolysaccharide. Infect. Immun. 66, 2264–2271.
[19] Jersmann, H.P.A. (2005) Time to adandon dogma: CD14 is expressed by non-myeloid lineage cells. Immunol. Cell Biol. 83, 462–467.
[20] Lloyd, K.L. and Kubes, P. (2006) GPI linked endothelial CD14 contributes to the detection of LPS. Am. J. Physiol. Heart Circ. Physiol. 291, H473–H481.
[21] Antal-Szalmas, P. (2000) Evaluation of CD14 in host defence. Eur. J. Clin. Invest. 30, 167–179.
[22] Triantafilou, M. and Triantafilou, K. (2005) The dynamics of LPS recognition: complex orchestration of multiple receptors. J. Endotoxin Res. 11, 5–11.
[23] Triantafilou, M. and Triantafilou, K. (2002) Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol. 23, 301–304.
[24] Devitt, A., Moffatt, O.D., Raykundalia, C., Capra, J.D., Simmons, D.L. and Gregory, C.D. (1998) Human CD14 mediated recognition and phagocytosis of apoptotic cells. Nature 392, 505–509.
[25] Gregory, C.D. and Devitt, A. (1999) CD14 and apoptosis. Apoptosis 4, 11–20.
[26] Petty, H.R. and Todd, R.F. (1996) Integrins as promiscuous signal transduction devices. Immunol. Today 17, 209–212.
[27] Tudor, K-S.R.S., Deem, T.L. and Cook-Mills, J.M. (2000) Novel of α4-integrin ligands on an endothelial cell line. Biochem. Cell Biol. 78, 99–113.
[28] Mould, A.P., Garratt, A.N., Askari, J.A., Akiyama, S.K. and Humphries, M.J. (1995) Identification of a novel ani-integrin monoclonal antibody that recognises a ligand-induced binding site epitope on the β1 subunit. FEBS Lett. 363, 118–122.
[29] Humphries, J.D., Schofield, N.R., Mostafavi-Pour, Z., Green, L.G., Garratt, A.N., Mould, A.P. and Humphries, M.J. (2005) Dual functionality of the anti-β1 integrin antibody, 12G10, exemplifies agonistic signalling from the ligand binding pocket of integrin adhesion receptors. J. Biol. Chem. 280, 10234–10243.
[30] Coe, A.P.F., Askari, J.A., Kline, A.D., Robinson, M.K., Kirby, H., Stephens, P.E. and Humphries, M.J. (2001) Generation of a minimal α5β1 integrin-Fc fragment. J. Biol. Chem. 276, 35854–35866.
[31] Hazeki, K., Masuda, N., Funami, K., Sukenobu, A., Matsumoto, M., Akira, S., Takeda, K., Seya, T. and Hazeki, O. (2003) Toll-like receptor-mediated tyrosine phosphorlyation of paxillin via MyD88-dependent and -independent pathways. Eur. J. Immunol. 33, 740–747.
[32] Triantafilou, M., Miyake, K., Golenbock, D.T. and Triantafilou, K. (2002) Mediators of innate recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. J. Cell Sci. 115, 2603–2611.
[33] Leitinger, B. and Hogg, N. (2002) The involvement of lipid rafts in the regulation of integrin function. J. Cell Sci. 115, 963–972.
[34] Beekhuizen, H., Blokland, I., Corsel-van Tilberg, A., Koning, F. and van Furth, R. (1991) CD14 contributes to the adherence of human monocytes to cytokine-stimulated endothelial cells. J. Immunol. 147, 3761–3767.
[35] Kobe, B. and Kajava, A.V. (2001) The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. 11, 725–732.
[36] Guidetti, G., Bertoni, A., Viola, M., Tira, E., Balduini, C. and Torti, M. (2002) The small proteoglycan decorin supports adhesion and activation of human platelets. Blood 100, 1707–1714.
[37] Zarewych, D.M., Kindzelskii, A.L., Todd, R.F. and Petty, H.R. (1996) LPS induces CD14 association with complement receptor type 3, which is reversed by neutrophil adhesion. J. Immunol. 156, 430–433.
[38] Uff, S., Clemetson, J.M., Harrison, T., Clemetson, K.J. and Emsley, J. (2002) Crystal structure of the platelet glycoprotein Ibα N-terminal domain reveals an unmasking mechanism for receptor activation. J. Biol. Chem. 277, 35657–35663.
[39] Riedemann, N.C., Guo, R-F. and Ward, P.A. (2003) Novel strategies for the treatment of sepsis. Nat. Med. 5, 517–524.