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New Assay to Detect Low Affinity Interaction and Characterisation of Leukocyte Receptors for Collagen including LAIR-1

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Summary

Leukocyte activity is controlled by numerous interactions between membrane receptors and ligands on the cell surface. These interactions are of low affinity making detection difficult. We developed a sensitive assay that could readily detect extremely weak interactions such as that between CD200 and the activating receptor CD200RLa ($K_d > 500 \mu\text{M}$) at the protein level. We used the new technology to screen for interaction of inhibitory receptors for collagens. We confirmed that both human and mouse leukocyte-associated immunoglobulin-like receptor (LAIR-1) and in addition the related inhibitory leukocyte immunoglobulin-like receptor subfamily B member 4 (Lilrb4, CD85K, Gp49B), bound collagen specifically whereas other cell surface proteins gave no binding. The monomeric affinities of the interactions were then determined to allow comparison with other leukocyte interactions and indicate conditions when this interaction might lead to inhibitory signals.

Keywords

inhibitory receptor; collagen; LAIR-1; surface interaction and immunoregulation

Introduction

Leukocyte activity is controlled by the interactions of its surface proteins with both soluble factors such as cytokines and chemokines, and ligands on other cells. Thus identification of ligands is essential in understanding the role of the receptors in regulation. However, the interactions of cell surface proteins with other cell surfaces tend to be of very low affinity when determined using isolated recombinant proteins corresponding to the extracellular regions of the proteins. For instance typical affinities for such interactions are around $2 \mu\text{M}$ (CTLA4/CD80 $K_d = 2 \mu\text{M}$, SIRP α /CD47 $K_d = 2 \mu\text{M}$, CD2/CD58 $K_d = 10 \mu\text{M}$, CD200/CD200R $K_d = 2 \mu\text{M}$) [1-6] with fast off rates usually giving half lives of the order of one second. Some highly functionally significant interactions have even lower affinities such as for CD8 $\alpha\alpha$ to MHC class I molecule HLA-A2 ($K_d \sim 200 \mu\text{M}$) [3]. Thus in order to detect binding to cells, recombinant proteins corresponding to the extracellular regions are often made multivalent for example by fusing to the Fc regions of IgG giving divalent protein, the Fc from IgM giving decavalent protein or by attaching the protein onto beads making a very avid binding reagent (discussed in [7]). These proteins, of various valencies, could be

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adapted and applied to screen for cells expressing receptors by flow cytometry and protein microarray [5, 8, 9]. With the ease of producing panels of recombinant proteins a powerful way of identifying new interactions is by screening at the protein level as illustrated by Eaton and co-workers using libraries of Fc fusion proteins [10].

In the present study we developed a highly sensitive bead binding assay capable of detecting exceptionally weak interactions using immobilised proteins and surface plasmon resonance (SPR). This is the combination of a conventional binding assay using SPR with the screening reagent – multivalent nanoparticles – taking advantage of the avidity of both reactants. Using the new assay we were able to examine the weak interaction between collagen and leukocyte membrane proteins. The recent identification of a motif in collagen as a ligand for the inhibitory leukocyte-associated immunoglobulin-like receptor (LAIR-1) is intriguing as it suggests that collagens could have immunoregulatory roles by interacting with this inhibitory receptor [11, 12]. LAIR-1 is composed of a single immunoglobulin superfamily (IgSF) extracellular domain, a transmembrane region and a cytoplasmic region containing ITIM (immuno-receptor tyrosine-based inhibitory motifs). LAIR-1 is expressed on the majority of peripheral blood mononuclear cells and thymocytes in mice and humans [13]. Collagens are the most abundant proteins in the body with major roles in the extracellular matrix of mammalian cells, either as network-forming or fibrillar molecules. They are intimately involved in cell adhesion, migration and differentiation [14, 15]. In humans, numerous diseases result from mutations in collagen genes [16]. In addition to structural roles, collagens interact with many cell membrane proteins, such as during cell adhesion to collagen in tissues, cell migration through collagen-rich stroma in normal differentiation and abnormal metastasis and in cell interactions in hemostasis [17, 18]. Fewer interactions have been reported between leukocytes and collagen as might be expected given the requirement of leukocytes to respond to the invasion by pathogens such as bacteria and viruses. In this paper, we examine in detail the interaction of mouse and human LAIR-1 with collagen and test the possibility that other ligands might exist for LAIR-1 and be present in the family of CD85 proteins using the new assay.

Results

Detection of low affinity interactions with a new assay using multivalent beads and BIAcore™ SPR analysis

In order to increase the sensitivity of detection of weak interactions such as those between leukocyte membrane proteins, the previously used bead assay was modified [7]. Recombinant proteins corresponding to the extracellular regions of the leukocyte membrane proteins were transiently expressed and immobilized via a tag [19] that can be biotinylated, to beads coated with avidin to provide a highly avid binding reagent that enabled the recognition of new interactions by binding to cells [9, 20]. We now combined these beads with SPR detection to identify interactions with leukocyte recombinant proteins. The assay was established using a known interaction at the limit of detection using monomeric recombinant proteins namely the interaction between mouse CD200 and CD200RLa (CD200R4) that has a K_d greater than 500 μM [21]. These and subsequent experiments were carried out at 37°C to exclude any effects due to the use of non-physiological temperature. Fig. 1 shows mCD200/rCD4d3+4 coupled beads passed over mCD200R (moderate affinity), mCD200RLa (very low affinity) and mCD200RLb (no binding) immobilised on the chip. Strong binding and roughly equivalent binding was obtained from both mCD200R ($K_d \sim 4 \mu\text{M}$) and mCD200RLa ($K_d > 500 \mu\text{M}$) with minimal binding to control mCD200RLb/rCD4d3+4 proteins. This contrasts with the good binding of monomeric mCD200 to mCD200R but barely detectable binding of CD200 to mCD200RLa as studied previously [21]. In addition the dimeric mCD200-Fc fusion proteins have failed to detect mCD200RLa on cells and in the BIAcore [21]. Thus the use of the multivalent binding reagent to

multivalent immobilised proteins clearly provides a highly sensitive assay capable of detecting extremely weak interactions – for comparison the monomeric affinity of MHC Class I for CD8 is in the region of 200 μM [6]. The almost complete lack of dissociation at 37°C even for the beads detecting the extremely low affinity mCD200-mCD200RLa binding (Fig. 1) indicates that the interaction must be highly polyvalent. Whilst not providing a quantitative assay it allows ready detection of weak interactions prior to more quantitative analysis. The use of transiently expressed protein coupled directly to avidin coated beads obviates the need for low pH elution steps in affinity purification steps minimising protein denaturation that can give anomalous binding in these types of assays.

Detection of the interaction of LAIR-1 with collagen I using the new assay

The reported interaction between LAIR-1 and a motif present in collagens was surprising as collagens are so widely distributed and LAIR-1 is an inhibitory receptor [12]. To confirm the LAIR-1/collagen interaction, the new bead/BIAcore™ assay described above was used. Human collagen type I (collagen I) was directly immobilized on the chip and beads coated with hLAIR-1/rCD4d3+4 were applied (Fig. 2). Even at a concentration as low as 0.004% w/v in the presence of 1% BSA in HBS running buffer, these beads bound strongly to the collagen I (solid line – 2700 RU responses) compared to negligible binding to control rCD4d3+4 and slight binding to LAIR-1 itself (see below). Note there is a strong bulk effect during the injection due to the signal from the BSA included to minimise any possible non-specific effects reflected by a constant signal during the injection. This confirms the previous report identifying collagen I as a ligand for LAIR-1 from cDNA library screening [12].

Although it is hard to calculate precisely the coverage of hLAIR-1/rCD4d3+4 on the bead, that is, the valency of the beads, the average concentration of hLAIR-1/rCD4d3+4 protein in the flow could be estimated to be 1.5 nM by inhibition ELISA standardized with rCD4d3+4 mAb OX68. The use of the multivalent bead is advantageous in amplifying such specific interactions with a requirement for minimal amount of protein.

Monomeric interactions of leukocyte membrane proteins with collagen I

The interaction between LAIR-1 and collagen has been described as high affinity [11, 12], but the measurements were made with dimeric protein and not directly comparable to the affinities measured for other interactions measured with monomeric proteins. Recombinant monomeric human and mouse LAIR-1 proteins were produced as monomeric protein with domain 3+4 of rat CD4 as a tag as used in other studies [7]. The affinity of LAIR-1 to collagen I was measured by SPR and in addition a synthetic collagen repeat previously shown to bind LAIR-1 was tested together with control non binding peptide [22]. Clear binding was obtained between mLAIR-1 and both (GPO)₁₀ and collagen I but minimal to the control peptide (GPP)₁₀ and also streptavidin (Fig. 3A). The dissociation constants K_d for mouse LAIR-1 binding to (GPO)₁₀ and collagen I were calculated to be 10 and 18 μM respectively (Fig. 3B and C). The similarity in affinities between collagen I and the synthetic peptide indicates that the trimeric repeat is an excellent structural mimic of the native collagen and compatible with the finding that LAIR-1 can bind several different kinds of collagen in its family, such as human collagen type III [11, 12, 22]. The human LAIR-1 gave a similar affinity ($K_d = 26 \mu\text{M}$) with collagen as the mouse LAIR-1 (Fig. 3D).

As LAIR-1 is present in a gene cluster on human chromosome 19q3.3 including several other receptors that are structurally related - the CD85 proteins, it seemed possible that additional collagen binding proteins might be found in the CD85 family. Fig. 2E shows that monomeric mLilrb4/rCD4d3+4 also bound to collagen I coated flow cells although the

interaction was weaker ($K_d = 39 \mu\text{M}$) than that of LAIR-1. Similarly, the affinity of mLilrb4/rCD4d3+4 to (GPO)₁₀ was $K_d = 33 \mu\text{M}$ (data not shown).

The possibility that collagens could interact with many surface proteins and that the interaction was some type of non-specific effect was tested by screening other cell surface proteins for binding to collagen I. Two other membrane proteins containing IgSF domains were tested in the same way with recombinant proteins. mCD200 and hSIRP α gave no detectable binding to collagen I or collagen related peptides (Fig. 4A and B). Other proteins such as human and rat CD200 also failed to give binding (data not shown) indicating that the collagen binding is not a broad non-specific effect common to many proteins with IgSF domains or due to the tag (note these experiments used either HIS tag or a CD4d3+4 tag in contrast to the published data on LAIR-1 binding that used Fc fusion proteins [11, 12]). The interaction of LAIR-1 with collagen I could also be detected in the presence of human serum (data not shown), suggesting that the interaction could occur in body fluids. Thus the LAIR-1 and Lilrb4 clearly bind specifically to collagen I although the latter binding is weaker.

Specific binding between LAIR-1 to Lilrb4

Because LAIR-1 and Lilrb4 are structurally related and members of a group of linked genes, it seemed possible that other interactions might occur between members of the CD85 gene cluster. The paradigm for this is the CD2/SLAM gene cluster of IgSF membrane proteins where several members interact with each other or homophilically [23, 24]. Weak binding between LAIR-1 and Lilrb4 was detected and characterised in both mouse and human by the qualitative multivalent SPR screening assay (Fig. 5). hLAIR-1/rCD4d3+4, mLilrb4/rCD4d3+4, mCD200RLa/rCD4d3+4 and rCD4d3+4 were immobilized on the four flow cell surfaces via biotin-streptavidin. Three types of multivalent beads coated with hLAIR-1, mCD200 and mLilrb4, were injected over the four flow cells. The hLAIR-1 coated beads gave significantly stronger binding to mLilrb4/rCD4d3+4 than to the other three flow cells. However the binding is weak when compared to the control weak interaction between mCD200 and mCD200RLa ($K_d > 500 \mu\text{M}$). The experiment was repeated in the reverse orientation with multivalent mLilrb4 beads binding to both Lilrb4 and LAIR-1 coated surfaces. The binding of mLilrb4 beads to hLAIR-1 (250 RU) was not as strong as the reverse interaction between hLAIR-1 beads and mLilrb4 (2500 RU). We attribute this to the different properties of the beads. For example, the mLilrb4 protein has larger dimensions than hLAIR-1 and is more glycosylated which may result in less molar coverage of the mLilrb4 coated particles. In addition mLilrb4 beads tend to bind more to hLilrb4 or mLilrb4 coated surface (1750 RU) than to hLAIR-1 or mLilrb4 (250 RU), as shown in the inset table of Fig. 5. This suggested that Lilrb4 could be homophilic across species. Further experiments compared the binding of hLAIR-1 to all the other members of CD85 family (CD85, CD85D, CD85E), which confirmed that Lilrb4 has the highest binding to human and mouse LAIR-1 (data not shown).

The affinity of LAIR-1 binding to Lilrb4 was determined by SPR. A series of concentrations of recombinant monomeric hLAIR-1/rCD4d3+4 was passed over hLilrb4/rCD4d3+4 and hLAIR-1/rCD4d3+4 (negative control) immobilized on the BIAcore™ (Fig. 6). Only a weak interaction between hLAIR-1 to hLilrb4 at the limit of the assay was found and a dissociation constant $K_d > 200 \mu\text{M}$ was estimated (Fig. 6), consistent with the result with the beads in Fig. 5.

Discussion

Several transmembrane receptors that recognise the typical collagen triple helix have the potential to signal. These include integrins, discoidin domain receptors (DDR1 and DDR2),

Glycoprotein VI (GPVI) and LAIR-1 (reviewed in [14]). Collagen binds to preformed DDR dimers on the cell membrane, and according to biophysical assays it binds only to dimeric DDRs with high affinity but not the monomeric DDR proteins [25]. Recently the binding motifs for DDR2 in collagen triple helix were identified [26]. The GPVI-collagen interaction is much weaker than the integrin-collagen interaction. Dimeric GPVI-Fc protein bound collagen and (GPO)₁₀ with dissociation constants K_d of 0.6 and 5 μ M respectively, whilst monomeric GPVI protein displayed almost no binding to collagen by SPR although some binding was found by plate binding assay [27-29]. Recently, it was found that LAIR-1 is also a receptor for collagen and that the dimeric LAIR-1-Fc protein binds collagen and (GPO)₁₀ with nM affinity [12]. So far most of the quantitative biophysical assays on collagens as above were detected with its receptors in the form of dimeric Fc fusion proteins. In this study we measured the affinity of monomeric LAIR-1 to collagen I showing it to be relatively weak but not dissimilar to many other cell-cell interactions [1-6]. It is significantly stronger than the GPVI interaction that is undetectable with monomeric protein. Thus this affinity is sufficient to explain the functional data on LAIR-1/collagen. These include showing that LAIR-1 transfected cells associated with collagen XVII transfected cells, that the binding of LAIR-1 Fc fusion protein to collagen expressing cells could be blocked by collagenase and purified collagen and that collagen could induce the inhibitory signal in LAIR-1 transfected reporter cells in both human and mouse [11, 12]. The biochemical studies described above support the immunological importance of the weak affinity ligand-receptor binding pair between collagen and LAIR-1.

We also identified a second member of the CD85 cluster, LILRB4 (CD85K), that can bind collagen I albeit weaker than LAIR-1. The finding that other cell surface proteins did not bind collagen I under these conditions suggests that the phenomenon is not due to non-specific stickiness. LILRB4 also showed some weak binding to LAIR-1 in both human and mouse species, but the significance of this interaction is difficult to evaluate because the LILRB4 proteins also showed some tendency to bind to themselves. The binding of LILRB4 and LAIR-1 to collagen distinguished these receptors from the other members of CD85 family, such as CD85D and CD85, which have been reported to bind HLA-G and classical MHC class I molecules [30, 31].

LILRB4 has been shown to interact with the integrin $\alpha_v\beta_3$ although the interaction has not been established using isolated proteins [32]. It is possible that this interaction was mediated by collagen as the collagen was found to bind many members of integrin family ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$) [33-35] and in some *in vitro* assays, $\alpha_v\beta_3$ also appears to be able to interact with collagen although this interaction may involve RGD motifs in denatured or partially unfolded collagen chains [36-38]. LAIR-1 has a higher affinity for collagen I (K_d = 26 μ M for hLAIR-1 and 18 μ M for mLAIR-1) than LILRB4 (K_d = 38 μ M). In the platelet adhesion scenario there was a similar situation where both GPVI and integrin bind collagen with different affinities [39-41]. Later it was found that GPVI and integrin act in a highly cooperative and integrated fashion to mediate the firm cell adhesion at the site of platelet-collagen contact [42, 43]. This suggests that collagens may interact with a variety of receptors including inhibitory receptors on the membrane surface.

The results in Fig. 5 implied that there could be specific interaction between LAIR-1 and LILRB4 and the monomeric binding assay in Fig. 6 indicated a K_d greater than 200 μ M. It seems reasonable to consider these proteins together as closely related IgSF members can interact among themselves with the best example in the CD2/SLAM family of receptors that interact with each other and probably evolved from a primordial homophilic interaction [44]. Both LAIR-1 and LILRB4 are present in a gene cluster on human chromosome 19q3.3 and are structurally related as shown in the sequence alignments in Fig. 7.

In conclusion, a new screening assay has been developed combining the advantage of surface plasmon resonance (SPR) with the innovative multivalent beads, onto which the relevant recombinant proteins are attached through different tags. One important aspect of the assay is that the proteins are immobilised directly from transient expression with no purification step involving denaturation that could give some denatured protein that may give anomalous binding. This assay enabled us to identify the weak interactions on leukocyte membrane surface whose affinity was too low to detect previously. Through this method, it was found that both the leukocyte membrane inhibitory receptors, LAIR-1 and LILRB4 (CD85K), show specific binding to collagen with different affinities, in addition to their weak affinity to each other. Although weak, the interaction may have significant functional effect in the regulation of immunological responses [12]. This interaction with inhibitory receptors also complements those characterised between collagen and both integrins and GPVI. One feature that distinguishes the LAIR-1 interaction from the well characterised interactions of collagen with integrins is that LAIR-1 is a very small cell surface protein with a single IgSF domain and a short hinge region, so interactions with collagen will require much closer proximity of the cell with collagen than for example with integrins. It is possible that any inhibition through LAIR-1 will depend on rearrangement of cell surface proteins in synapse-like regions and collagen inhibition of leukocyte activity may be only occur in restricted circumstances.

Materials and methods

Construction, expression and purification of soluble recombinant proteins

Constructs for the human and mouse LAIR-1 and LILRB4 were prepared from cDNA clones from Geneservice Ltd (UK) by inserting the sequence for the extracellular domains into the pEF-BOS vector [45] together with the sequence for domains 3 and 4 of rat CD4 (rCD4d3+4) and sequence to permit biotinylation by biotin ligase BirA (Avidity LLC, Colorado, US) near the C terminus [9]. The boundary between the hLAIR1 part and CD4 was AEGAPSTSIT, for mLAIR1 SDTSWSTSIT, for hLILRB4 SGLRRSTSIT and for mLILRB4 ETKDQSTSIT (CD4 linker is underlined). The pEF-BOS constructs were transfected into HEK 293T cells with reagent PEI (polyethylenimine, linear MW ~25,000, Polysciences, Inc.) at the DNA:PEI ratio of 1:10 [46]. The cells were incubated in serum free Xvivo10 medium (BioWhittaker, Walkersville, MD, USA) for 5 days at 37°C, the supernatants were collected and concentrated, assayed by an inhibition ELISA using OX68 mAb (specific for the rCD4d3+4 tag) to measure the expression level of recombinant proteins [21]. The yield of the protein was 5 - 20 µg/ml. The protein was biotinylated, dialyzed in PBS and coupled to avidin coated yellow fluorescent beads (SPHERO™, 0.46 µm diameter, Spherotech Inc. Libertyville, IL) as described previously [5, 8].

For monomeric binding assays where larger amounts of protein were needed, stable cell lines were produced using the pEE14 vector (Lonza Biologics, Slough, UK) and Chinese hamster ovary (CHO) cells as described. The recombinant protein was purified by affinity chromatography with rat CD4d3+4 mAb, OX68 Sepharose 4B column, eluted with 0.1 M glycine-HCl buffer (pH 2.5) and subsequent gel filtration through a Superdex S-200 HR 10/30 column (Pharmacia, Uppsala, Sweden) to exclude the large aggregates. The yield of purified proteins was between 0.2 and 1 mg/l.

Detection of low affinity interactions and measurement of affinities using surface plasmon resonance (SPR)

Affinity and kinetic data were collected using a BIAcore 2000 (Biacore™) at 37°C as previously described [21]. Streptavidin (~2000 Response Units (RU)) was coupled to a CM5 research grade chip using amine coupling. Biotinylated recombinant proteins and control

rCD4d3+4 were each immobilized at about 2000 RU. For kinetic analysis of monomeric binding, serially diluted monomeric purified proteins, such as hLAIR-1/rCD4d3+4, mLAIIR-1/rCD4d3+4, hLilrb4/rCD4d3+4 or mLilrb4/rCD4d3+4 were injected at the indicated concentrations over all four flow cells connected in series. The theoretical molecular extinction coefficients of 38,010, 49,390, 64,850 and 78,100 M⁻¹cm⁻¹ respectively were calculated. Dissociation constant K_d values were obtained by nonlinear curve fitting to the equilibrium binding data. For the qualitative binding assay of multivalent beads to immobilized proteins, fresh samples were prepared before each experiment. 10 µl 0.1% w/v (in PBS buffer) avidin coated beads were incubated with dialyzed biotinylated recombinant proteins for 3 hours, followed by centrifuging (10 min at 13,000rpm), removal of supernatant and resuspension of the beads in 250 µl HBS buffer (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) with 1% bovine serum albumin (BSA). 10µl diluted bead suspension was injected over the flow cells at 1-2 µl/min with HBS pH 7.4 as running buffer at 37°C. The results shown are representative of at least three experiments.

Acid-soluble human collagen type I (from human placenta, C7774, Sigma, UK) was immobilized (500–2000 RU) on a CM5 biosensor chip using amine coupling. Immobilized triple-helical peptides composed of GCO(GPO)₁₀GCOG-NH₂ ((GPO)₁₀, also known as collagen-related peptide or CRP) and GCP(GPP)₁₀GCPG-NH₂ ((GPP)₁₀) [22] were immobilized (~2000 RU) using a cysteine coupling kit, according to the manufacturer's instructions [47].

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Abbreviations

LAIR-1	leukocyte-associated immunoglobulin-like receptor 1
SPR	surface plasmon resonance
IgSF	immunoglobulin superfamily
Lilrb4	leukocyte immunoglobulin-like receptor subfamily B member 4

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Figure 1.

Sensitive assay to detect low affinity interactions using beads expressing multivalent ligands to immobilised proteins detected by SPR. Three flow cells were coated with ~2000 RU mCD200R, mCD200RLa and mCD200RLb, which respectively have moderate, very low and no affinity for monomeric mCD200 [21]. When the multivalent mCD200 coated beads were injected at 2 μ l/min at 37°C, mCD200RLa gave as strong binding to mCD200 as mCD200R (>3000 RU). No binding of mCD200RLb to mCD200 was detected and only the signal due to the analyte itself is seen.

Figure 2. hLAIR1/rCD4d3+4 beads bound to collagen but not to control protein. Collagen (1000 RU), hLAIR1/rCD4d3+4 (2000 RU) and rCD4d3+4 (1500 RU, negative control) were immobilised, and hLAIR-1/rCD4d3+4 beads passed over. The figure shows responses from the three flow cells superimposed.

Figure 3.

Binding of LAIR-1 and Lilrb4 to collagen or collagen related peptide. (A) Soluble monomeric mLAIR-1/rCD4d3+4 (43 μM) was applied to collagen (2000 RU), collagen related peptide trimer, (GPO)₁₀ (2000 RU), control peptide trimer (GPP)₁₀ (2000 RU) and negative control streptavidin (1000 RU) coated flow cells. (B, C) Binding curves for experiments as in (A) but varying the concentration of purified mLAIR-1/rCD4d3+4. K_d of 10 and 18 μM were calculated by nonlinear curve fitting of the Langmuir isotherm (line) to the binding data of monomeric mLAIR-1/rCD4d3+4 to (GPP)₁₀ and collagen with the values for the negative control (GPP)₁₀ subtracted. (D) and (E) Comparable experiment to (B) and (C) showing hLAIR-1/rCD4d3+4 and mLilrb4/rCD4d3+4 bound to collagen with K_d = 26 and 39 μM respectively.

Figure 4.

CD200 and SIRPα do not bind collagen. Soluble monomeric mCD200/rCD4d3+4 (9.3 μM) (A) and hSIRPα (26 μM) (B) were applied to the collagen related peptide, (GPO)₁₀, collagen, control peptide (GPP)₁₀ and negative control streptavidin coated flow cells. The X-axis indicates the duration of injection of ligand in seconds. The binding was indistinguishable to the control with only the bulk effect due to the high concentrations of protein in the analyte.

Figure 5.

hLAIR-1 gives comparable bead binding to mLiIrb4 as that between mCD200 and mCD200RLa. mLiIrb4/rCD4d3+4, mCD200RLa/rCD4d3+4, hLAIR1/rCD4d3+4 and rCD4d3+4 were immobilized on four flow cells and then three conjugated sets of beads were injected sequentially. (A) hLAIR-1 beads gave strong binding to mLiIrb4 but not mCD200RLa or control rCD4d3+4 with traces on hLAIR-1. (B) mCD200RLa beads gave strong binding to its ligand CD200 but not the other proteins. (C) mLiIrb4 beads gave minimal binding to mCD200 but very weak binding to the other proteins including the control protein. Inset table: the amount of multivalent beads coated with hLair1 and mLiIrb4 that bound to surfaces with immobilized hLair1, mLiIrb4, hLair1 and mLiIrb4 from another experiment are listed.

Figure 6.

The interaction between hLAIR-1 and hLilrb4 is very weak. Y-axis indicates the equilibrium binding of soluble monomeric hLAIR1/rCD4d3+4 to immobilized hLilrb4/rCD4d3+4 (2500 RU), as set up in Fig. 3. Despite the high concentration of protein used only minimal binding was observed. Thus only an approximate dissociation constant K_d of $> 200\mu\text{M}$ could be estimated from the nonlinear curve fitting shown by the dotted line.

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mLAIR-1 MS LHPYI LLVLC LGWKINT QEGSLPDLTF PNSSLM SQGTFFVT VVCSYSOKHDLY
hLAIR-1 MS PHPTALLGLVLC LAQTIHTQEEQLPRLSLSKEPGTIV PLQSHVTFVCRGPVGVQTF
hLilrb4 MI PTFTTALLCLGLSLGPRDTMQAGLPKPTLWAEFGSVISWGNSTI WCQGTLEAREY
mLilr4 MI AMLTIVLLYLGIL LEPRTA VQAGHLPKPI QWAEFGSVI AAYTSMT WCQGSWEAQY

mLAIR-1 NMVRLK KDGSTFMEKS - -TEPYKTEDEFETGPVNETITGHVSCIVYSKGITWSEHSKT
hLAIR-1 RLEERSSTYNDT EDVSQASPESEARFRIDSVSEGNAGPYRCIYYKPPKWSEOSDY
hLilrb4 RLDKEESPAPWDRQNP - -LEPKNKARFSIPSMTEGYAGRYRCYRSPVGVWSQSDP
mLilr4 HLYKEKSVNPWDTQVP - -LETRNKAKENLPSSMTTSYAGIYKCYVESAAAGFSEHSDA

mLAIR-1 LELKVIKEN - - - - -VI QTPAG - - - - -
hLAIR-1 LELLVKETSGGPDSPDTEPGSSAG - - - - -
hLilrb4 LELVMTGAYSKPTLSALPSPLVTS GKS VTL L CQSRSPMDTFL LI KERA AHPLHLRSE
mLilr4 MELVMTGAYENPSLSVYPSSNVTSGVSI SFSCSSSI VFGRFIL IQEGKHGLSWTLD SQ

mLAIR-1 - - - - -PTSDTS - - - - -WK TYS - - - - -
hLAIR-1 - - - - -PTQRPSDN SHNEHAPASQGLKAEH - - - - -
hLilrb4 HGAQQ- HQAEFPMSPVTSVHGQTYRCFSSHGF SHYL SHPSDPLELIVSGSLEGP RP S
mLilr4 HQANQPSYATFVLDAVTPN HNGTFRCYGYFRNEPQVWSKPSNSLDLMI S - - - - -

mLAIR-1 - - - - -LYFTVVS VIFLCIS-ALFCFLRH
hLAIR-1 - - - - -LYILIGVSVVFLFC LLLVLFCLHRQ
hLilrb4 PTRSVSTAAGPEDQPLMPTGSGVPHSGLRRHWEVLI GVLVVSILL SLLFL LQHWRQ
mLilr4 - - - - -ETKDQSSSTPT - - - - -EDGLETYQKIL L GVLVSFLL FFF LLLF LLI GYQY

mLAIR-1 RQK K - - - - -QGLPN - - - - -NKRQQRP P
hLAIR-1 NQIK - - - - -QGPPR - - - - -SKDEEQK P
hLilrb4 G - - - - -KHRTL AQRQADFQRPPGA AEPEPKDGG LQRRSSPAADVQGENFCAA VKNTP P
mLilr4 GHKK - - - - -AN - - - - -ASVKNTQSE

mLAIR-1 ERLN ATNG - -LEMTDPDI VADRLPE-DRWTET - - - - -WTP - - - - -
hLAIR-1 QRPDLAVDV - -LERTADKATVNGLE KDR ETD - - - - -SAL - - - - -
hLilrb4 DQVEIDTRQSPHSEDPQAVTYAKVCH- SRPRREMASPPSPLSGEFLDKDRQAEEDRQ
mLilr4 NNAELNSWNPQNE DPQGI VYAQVKP - -SR LQK - - - - -DT - - - - -

mLAIR-1 - - - - -VAGDLQEVTYLCLDHHSITGRAVGAVTSQSTD- MR ESSTYAAIIR R
hLAIR-1 - - - - -AAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMAL - - - - -
hLilrb4 MDTEAAASEAPQEVTYAQLHSFTLRQKATEPPPSQEGASPAEPSVYATLAIH
mLilr4 - - - - -ACKETQEVTYAQLCIRTQEQNNS - - - - -

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Figure 7.
Comparison of the sequences of hLAIR-1, mLAIR-1, hLilrb4 and mLilr4. Residues identical in 3 or 4 sequences are boxed.