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Incorporation of a Ligand Peptide for Immune Inhibitory Receptor LAIR-1 on Biomaterial Surfaces Inhibits Macrophage Inflammatory Responses

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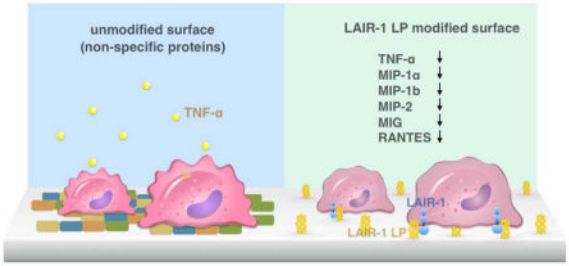
Abstract

Leukocyte-associated Ig-like receptor-1 (LAIR-1) is an inhibitory receptor broadly expressed on immune cells, with its ligands residing within the extracellular matrix protein collagen. In this study, we modify surfaces with a LAIR-1 ligand peptide (LP), and observe that macrophages cultured on LAIR-1 LP-conjugated surfaces exhibited significantly reduced secretion of inflammatory cytokines in response to proinflammatory stimuli that mimic an injured environment. These downregulated mediators include TNF-α, MIP-1α, MIP-1β, MIP-2, RANTES, and MIG. Knockdown of LAIR-1 using siRNA abrogates this inhibition of cytokine secretion, supporting the specificity of the inhibitory effect to this receptor. These results are the first to demonstrate that integration of LAIR-1 ligands with biomaterials could suppress inflammatory responses.

Graphical abstract



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Supporting Information

Supporting Information is available from the Wiley Online Library or from the authors.

Keywords

LAIR-1; immunomodulatory biomaterial; inflammation; collagen peptide; macrophage

Implantable biomedical devices, including biosensors, cardiovascular devices, orthopedic implants, and tissue engineering scaffolds, are key components of modern medicine.[1] However, one of the major challenges associated with implanted devices is that biomaterials elicit host immune responses including acute/chronic inflammation, a foreign body reaction, and fibrotic capsule formation, leading to isolation from the host and loss of functional interaction of the device with the surrounding tissue.[1a, 2] In addition, adverse immune reactions may interfere with healing, and cause pain, inflammation, or tissue destruction.[3] Thus, improving the interaction of biomaterials with the host immune system is needed to achieve successful biological performance of implanted devices.

Extensive efforts in designing biomaterials are under investigation to mitigate material-induced host inflammatory responses.[4] An emerging strategy in biomaterial design leverages direct molecular interactions with immune cells, for example using biomolecules that bind to and interact with local immune cells, modulating their function. Previously, we reported that modifying biomaterials with CD200, an endogenously expressed self-associated protein, altered pro-inflammatory immune responses.[5] We demonstrated that CD200 modification reduced the secretion of pro-inflammatory cytokines of macrophages, and reduced infiltration of immune cells and reactive oxygen species generation in the tissue surrounding subcutaneously implanted beads.[5b] Stachelek *et al.* used CD47 to modify polyurethane (PU) and polyvinylchloride films, and they demonstrated significantly reduced attachment of inflammatory cells, resulting in less oxidative degradation after subdermal implantation when compared to unmodified materials.[6] These approaches utilized immunomodulatory ligands naturally expressed on the surface of cells, while extracellular matrix-based ligands remain unexplored.

Collagen is the most abundant extracellular matrix protein in the body, and it has been proposed that its interactions with the leukocyte-associated Ig-like receptor (LAIR-1, or CD305) prevent spurious immune activation. LAIR-1 is an immune inhibitory receptor broadly expressed on the surface of most immune cells, including monocytes/macrophages, natural killer cells, dendritic cells, T cells, and B cells.[7] It is a transmembrane protein containing a single extracellular Ig-like domain and two immunoreceptor tyrosine-based inhibitory motifs in its cytoplasmic tail.[7a] Antibodies that bind and cross-link LAIR-1 *in vitro* inhibit target cell lysis by NK cells,[7b] cytotoxic activity of effector T cells upon CD3 cross-linking, [8] and FcεR-induced degranulation of mast cells,[9] suggesting potent inhibitory signaling across many immune cell types. LAIR-1 is unique in that it binds to an extracellular matrix protein (collagen),[7a] while most known ligands of inhibitory immune receptors are cell-bound. The interaction of collagen with LAIR-1 on immune cells demonstrated direct inhibition of immune cell activation *in vitro*.[7, 10] Furthermore, tumor cells overexpressing collagen have been reported to inhibit NK cell cytotoxicity through the LAIR-1/collagen interactions,[11] showing a potential target for anti-cancer therapeutics. Decreased LAIR-1 expression has also been measured in patients with the autoimmune

disease rheumatoid arthritis, and suggests that chronic inflammation is linked to this receptor.[10c]

Although the inhibitory effects of the collagen/LAIR-1 interaction on immune cells have been increasingly examined, its utilization for decreasing inflammation in response to biomaterial surfaces has not yet been investigated as a materials design strategy. In this study, we engineer materials that leverage the immunomodulatory properties of collagen by utilizing a peptide based on collagen (peptide III-30) (Figure 1a) that was identified from a library of overlapping collagen III peptides to potently bind to human LAIR-1.[12] It showed high binding to LAIR-1 expressing cells, and inhibited CD3-induced T cell activation and FcεR1-induced degranulation of mast cells via LAIR-1.[12] Based on these previous studies, we designed a LAIR-1 ligand peptide (LP) containing the LAIR-1 binding sequence from the collagen III, $(GPP)_5$ flanking regions at both ends to ensure stable triple helical conformation, and a N-terminal cysteine for surface conjugation, yielding the sequence $C(GPP)_5GAOGLRGGAGPOGPEGGKGAAGPOGPO(GPP)_5\text{-}NH_2$. Although the collagen peptide library was based on the human protein sequence and screened for adhesion to human LAIR-1, the collagen-binding site of LAIR-1 was reported to be well conserved in human and mouse.[10a, 10b, 13]

The secondary structure and folding properties of our synthesized LAIR-1 LP was determined by circular dichroism (CD) spectra, showing a positive peak at 225 nm and a negative peak at 196 nm, characteristic of triple-helical collagen-like structure[14] (Figure 1b). The crystallographic structure of the LAIR-1 binding site shows multiple simultaneous interactions with two of the three collagen strands, requiring an intact trimeric structure for binding [13]. Our observation of an immunomodulatory effect strongly suggests that the peptide structure remains triple-helical when immobilized on the surface. Our previous studies imaging recombinant collagen III (from which LAIR-1 LP is derived) using AFM on polystyrene substrates has also shown that the triple-helical structure of collagen stay intact and functional for cellular binding when the substrate is incubated at or below the protein's melting temperature, T_m [15]. The T_m of the peptide III-30 was measured to be at 42.9 °C [16], so our experiments are within this temperature limit.

To determine surface saturation concentrations of LAIR-1 LP conjugated to maleimide-activated surfaces, fluorescently labeled LAIR-1 LP was used. Fluorescence intensity was found to increase up to approximately 10–20 μg mL^{-1} of LAIR-1 LP loading concentration added to each well, above which the amount of LAIR-1 LP was saturated at the surface (Figure 1c). There was minimal binding of LAIR-1 LP to surfaces that lacked maleimide groups (Figure S1, Supporting Information)

To investigate the immune-inhibitory properties of LAIR-1 LP conjugated surfaces, we cultured mouse bone marrow derived macrophages (mBMDM) on the LAIR-1 LP-modified surface (20 μg mL^{-1} loading concentration), and stimulated with inflammatory activators lipopolysaccharide (LPS) and interferon gamma (IFN-γ). LPS/IFN-γ stimulation leads to activation of macrophages that is representative of inflammatory macrophages present in the damaged tissue surrounding surgical implantation of biomaterials, activating many of the same signaling pathways. After 18 h of culture, cell culture supernatants were collected, and

pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and anti-inflammatory cytokine interleukin-10 (IL-10) secretion were measured by ELISA. In addition, gene expression of both cytokines was measured by qRT-PCR (Figure S2, Supporting Information). First, we found that a LAIR-1 LP loading concentration of at least 10 μg mL^{-1} was necessary to maximally inhibit mouse macrophage activation, based on TNF-α secretion level from mBMDM (Figure 1d). These data are consistent with the peptide saturation data in Figure 1c. We found that for mBMDM activated with IFN-γ and LPS, the TNF-α secretion was significantly reduced by 60% for the LAIR-1 LP conjugated surface compared to the control surfaces (maleimide-active, cysteine-capped, and CSIINFEKL ovalbumin-conjugated surface) (Figure 2a), with a p-value < 0.01. As expected, this decrease in TNF-α is also observed for mBMDM cells on surface-adsorbed full-length recombinant collagen III, from which the LAIR-1 LP is derived (Figure S3, Supporting Information). Interestingly, when macrophages were incubated with unbound LAIR-1 LP, there was no decrease in TNF-α when compared to untreated cells, suggesting that immobilization of the peptide domain is required (Figure S4, Supporting Information). The level of anti-inflammatory cytokine IL-10 was similar between the cysteine-capped surface and LAIR-1 LP conjugated surface (Figure 2b). Gene expression levels measured by qRT-PCR showed no significant difference of TNF-α and IL-10 from mBMDM on the LAIR-1 LP conjugated surface, compared to the cysteine-conjugated control surface (Figure S2, Supporting Information); it is likely that the apparent discrepancy for TNF-α is due to the timepoint used, since TNF-α gene expression is early and transient after LPS stimulation [17].

Our LAIR-1 LP has its amino acid binding sequence derived from human collagen, so we wanted to examine whether results obtained by mouse BMDM could be recapitulated using human macrophages. Experiments were performed with human monocyte derived macrophages (hMDM) cultured on the LAIR-1 LP-conjugated surface and induced with LPS. While our data showed high donor-to-donor variability with respect to absolute values of TNF-α and IL-10 from the human primary cells, we still obtained consistent trends between each individual. We also observed that LAIR-1 LP induced a significant 60% reduction in TNF-α secretion by ELISA (Figure 2c) and obtained the same level of IL-10 (Figure 2d), relative to the control surface. Gene expression levels in hMDM also showed a significant decrease for TNF-α but the same amount of IL-10 gene expression, consistent with the ELISA results (Figure S5, Supporting Information).

To determine whether inhibition of macrophages on the LAIR-1 LP conjugated surface is caused by the interaction of LP with LAIR-1 on the macrophages, we knocked down LAIR-1 expression in mBMDM using siRNA. We achieved a 50–60% decrease of LAIR-1 expression in cells after transfection with Viromer Blue and LAIR-1 siRNA, when compared to cells transfected with non-target control siRNA (Figure 3a). LAIR-1 siRNA transfected cells and non-target control siRNA transfected cells were cultured onto maleimide-functionalized polystyrene surfaces that were conjugated with cysteine, CSIINFEKL, and LAIR-1 LP, and stimulated with LPS and IFN-γ for 18 h. As expected based on prior data (Figure 2a), TNF-α secretion was significantly reduced in cells transfected with the non-target control siRNA and incubated on the LAIR-1 LP conjugated surfaces, relative to incubation on the control surfaces (Cys and CSIINFEKL) (Figure 3b). However, TNF-α secretion by LAIR-1 knockdown mBMDM cells cultured on the LAIR-1 LP conjugated

surface was not significantly different from cells cultured on cysteine or CSIINFEKL-conjugated surfaces. Furthermore, the levels of TNF-α secreted by the LAIR-1 knockdown cells were significantly higher compared to the amounts secreted by cells transfected with the non-target control siRNA and cultured on LAIR-1 LP conjugated surface. These data demonstrate that reduction of pro-inflammatory cytokine TNF-α secretion in macrophages cultured on LAIR-1 LP modified surfaces is mediated by interactions through LAIR-1.

To examine the expression of additional pro-inflammatory cytokines, we comprehensively assessed mouse macrophage cytokine/chemokine secretion profiles by performing a multiplex analysis of a panel of 31 secreted inflammation-relevant proteins. mBMDM were cultured on LAIR-1 LP- and cysteine-conjugated surfaces and stimulated with LPS and IFN-γ, and supernatants were subjected to multiplex array analysis (Figure 4a). We found that many inflammatory cytokines/chemokines, including RANTES (regulated upon activation, normal T-cell expressed, and secreted; CCL-5), MIG (monokine induced by gamma interferon; CXCL-9), MIP-1α (macrophage inflammatory protein-1α; CCL-3), MIP-1β (CCL-4), and MIP-2 (CXCL-2), shared a similar trend with TNF-α and were significantly decreased in mBMDM cells cultured on the LAIR-1 LP conjugated surface relative to the cysteine-capped control surface (Figure 4b). Other cytokines, including KC (keratinocyte-derived chemokine; CXCL-1), G-CSF (granulocyte colony-stimulating factor), and IL-6, also demonstrated a decreased average on LAIR-1 LP, but additional experiments are needed to verify these trends. Overall, these results support the strategy of enhancing the therapeutic value of biomaterials by attachment of collagen-mimetic LAIR-1 ligands to their surfaces.

Our results showing decreased immune activation are consistent with other investigations involving LAIR-1 binding to alternative protein domains with collagen-like properties. Fraser *et al.* showed suppression of LPS-stimulated cytokine levels such as TNF-α, IL-1β and MIP-1α released by hMDMs on C1q bound surfaces.[18] C1q is also a LAIR-1 ligand, and binding of C1q's collagen-like region to LAIR-1 on monocytes has been shown to suppress immune cell activity by inducing LAIR-1 phosphorylation, inhibiting the differentiation of monocytes into dendritic cells, and inhibiting secretion of pro-inflammatory cytokines.[19] In addition, surfactant protein D, which has helical collagenous domains, was also reported to interact with LAIR-1 and suppress immune cell activity by inhibiting production of reactive oxygen species.[20]

The cytokines that are inhibited in our study are typically produced by tissue-resident or infiltrated immune cells (e.g., neutrophils, monocyte/macrophages) during inflammation caused by injury or implantation of a foreign material. TNF-α induces chemokine production, and stimulates expression of cell adhesion molecules on vascular endothelial cells, which facilitate extravasation of leukocytes from the bloodstream.[21] Chemokines released by activated inflammatory cells and endothelial cells orchestrate immune responses by guiding migration of leukocytes toward the injured tissue or implanted material and further advancing inflammation.[21b, 22] Of the chemokines that were secreted at lower levels by macrophages cultured on LAIR-1 LP conjugated surface, KC and MIP-2 are reported to recruit neutrophils,[23] and MIP-1α, MIP-1β, and RANTES chemoattract macrophages[24] and T cells.[25] IL-6 enhances T lymphocyte activation and proliferation,[25a] and MIG recruits NK cells and activated T lymphocytes.[26] Lymphocytes are reported to facilitate

macrophage fusion, and MIP-1β and TNF-α have been implicated in macrophage fusion to form foreign body giant cells.[25a]

Although immunomodulatory effects were also observed for the collagen III protein (Fig. S3), there are advantages towards using a LAIR-1 peptide rather than full-length collagen. For longer-term surface stability, chemical conjugation on the surface is preferred to prevent desorption. Amine-based reactions are often employed for this, but in collagen, there are numerous surface lysines in the triple-helical domain (e.g., 114 lysines for collagen III trimer), making specificity of conjugation difficult. In fact, a lysine exists in the LAIR-LP binding region, so surface conjugation could even inhibit LAIR-1 binding. Secondly, the ability to chemically synthesize peptide rather than rely on collagen (which normally comes from bovine) could avoid safety issues associated with animal sources when used in *in vivo* applications. Finally, full-length collagen has also been shown to host numerous other sites that interact with other cell receptors [27], and these ligand sites have been implicated in processes including integrin-mediated binding and cell differentiation [16, 28], platelet activation via the GpVI receptor [29], and activation of discoidin domain receptor 2 which regulates mammary tissue and bone development [29]. Therefore, using an isolated, defined LAIR-1 LP sequence could prevent non-intended biological effects of the functionalized surface.

Our results demonstrate that the interaction of collagen-derived LAIR-1 LP (immobilized on a surface) with immune inhibitory receptor, LAIR-1 (expressed on macrophages), significantly reduced their secretion of multiple pro-inflammatory cytokines and chemokines. This inhibition was mediated by the interaction of the peptide with LAIR-1 expressed by macrophages. To our knowledge, it is the first demonstration of conjugating a collagen-derived peptide to materials for modulating macrophages via binding to LAIR-1 inhibitory receptors. Our work shows both the specificity to LAIR-1, as well as the ligand's broad effects towards suppression of macrophage inflammatory responses. Since macrophages are thought to be a key player in material-induced inflammation and host response, the conjugation of LAIR-1 LP to engineered biomaterials may be a strategy to reduce local macrophage activation and ultimately lead to a more favorable tissue response toward implanted biomaterials.

Experimental Section

LAIR-1 ligand peptide (LAIR-1 LP)

LAIR-1 LP was purchased from Genscript (Piscataway, NJ, USA) and Prof. Richard Farndale's group (University of Cambridge, U.K.). The amino acid sequence of a single chain of the peptide is C$(GPP)_5$**GAOGLRGGAGPOGPEGGKGAAGPOGPO**$(GPP)_5$ with bold residues denoting the collagen III synthetic peptide III-30,[12] two $(GPP)_5$ flanking regions to ensure triple helical conformation of folded peptides, and a N-terminal cysteine for surface conjugation.

Circular dichroism

To verify the secondary structure of the synthesized LAIR-1 LP, circular dichroism (CD) spectra were acquired using a spectropolarimeter (Jasco 810) using methods previously described for recombinant collagen.[28a, 30] Briefly, 200 μg mL^{-1} of LAIR-1 LP in 45 mM sodium acetate was placed in a cuvette with path length of 0.1 cm, and scanned between 190–260 nm. CD data were an average of 3 scans, with the blank (buffer alone) subtracted.

Preparation of LAIR-1 LP-conjugated surfaces

LAIR-1 LP (with N-terminal cysteine) was diluted in binding buffer (0.1 M sodium phosphate, 0.15 M sodium chloride, 10 mM EDTA; pH 7.2) to 200 μg mL^{-1} (~40 μM) and incubated with 2 mM Tris(2-carboxyethyl)phosphine-HCl (TCEP) for 20 min at room temperature to reduce disulfide bonds. Binding buffer was further added to prepare 20 μg mL^{-1} peptide solution. Maleimide-activated polystyrene wells of 96-well microplates (Thermo Scientific) were washed with PBS (Lonza) and incubated with LAIR-1 LP overnight at 4 °C. Freshly prepared 40 μg mL^{-1} cysteine in binding buffer was syringe filtered, and 50 μL of cysteine solution was added to each well to deactivate unconjugated maleimide sites. After 1 h incubation, the plate was washed three times with PBS.

For surface characterization, LAIR-1 LP was fluorescently labeled by incubating with four times molar excess of Alexa Fluor 488 NHS ester (Thermo Scientific), relative to amine groups in the peptide, at room temperature for 2 h. Unreacted NHS ester was quenched by adding five times molar excess of 10 mM hydroxylamine solution in PBS. TCEP was then added to Alexa Fluor 488 conjugated LAIR-1 LP, and binding buffer was added to prepare 40 μg mL^{-1} peptide solution. 100 μL of fluorescently labeled LAIR-1 LP ranging between 0–40 μg mL^{-1} was incubated in wells of a maleimide-activated microplate (surface area of 0.33 cm^2 per well) at room temperature for 2 h with shaking, and washed twice with PBS containing 0.05% Tween-20 and twice with PBS. The fluorescence intensity at 517 nm emission with 494 nm excitation was measured (SpectraMax M2, Molecular Devices). Data were fitted by nonlinear regression to an exponential one phase decay curve using GraphPad Prism 6.

Mouse macrophage response in vitro

All procedures requiring animal tissues were carried out in accordance with protocols approved by the Institute for Animal Care and Use Committee at the University of California, Irvine. In order to obtain mouse bone marrow-derived macrophages (mBMDMs), femurs from 6 to 12 week old C57BL/6 mice (Jackson Laboratories) were harvested. Bone marrow was flushed using syringes with 26G needles, and treated with ACK lysis buffer (Thermo Fisher). Isolated cells were differentiated to mBMDMs, by culturing in DMEM (Life Tech) supplemented with 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 0.292 mg mL^{-1} L-glutamine, 10% heat-inactivated FBS, and 10% conditioned media containing macrophage colony-stimulating factor (M-CSF) for 7 days. M-CSF containing conditioned media is generated by culturing CMG 12–14 cells expressing recombinant M-CSF in alpha-MEM supplemented with 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 0.292 mg mL^{-1} L-glutamine, and 10% heat-inactivated FBS. On day 7 of growth, cells were dislodged using cell-dissociation buffer (Gibco) after washing with PBS. 100 μL of 5×10^4 mBMDMs

were seeded onto LAIR-1 LP-conjugated plate and cysteine-capped plate as a control. Two hours after cell seeding, 0.3 ng mL^{-1} LPS and 0.1 ng mL^{-1} IFNγ were added as stimulants. After 18 h, supernatant was collected for ELISA.

Human macrophage response in vitro

All blood samples were collected at the University of California, Irvine (UCI) Institute for Clinical and Translational Science in accordance with guidelines and approval of the UCI Institutional Review Board. Human peripheral blood monocytes were isolated from peripheral blood mononuclear cells (PBMCs) by countercurrent elutriation. Human monocyte derived macrophages (hMDM) were generated from monocytes by culture at 5×10^5 cells mL^{-1} for 7 days in RPMI1640 (Invitrogen), 10% defined FBS (Hyclone), 2 mM L-Glutamine and 1% penicillin/streptomycin (complete media) containing 25 ng mL^{-1} recombinant human M-CSF (rhM-CSF) (PeproTech) with addition of fresh media + rhM-CSF at day 3. The adherent hMDMs were harvested at day 7–8 by washing twice with 1xPBS and incubating with non-enzymatic CellStripper (CellGro) for 20 – 30 min. 100 μL of 5×10^4 hMDMs in Xvivo-15 media (Lonza)/1% L-glutamate was added to the LAIR-1 LP-conjugated plate and the cysteine-capped plate (control). After centrifugation for 3 min at 700 rpm at room temperature, hMDMs were incubated in 5% CO_2 air for 30 min. Macrophages were treated with or without 10 ng mL^{-1} ultrapure LPS (List Biological Laboratories Inc, Campbell, CA) for 2, 6 and 19 hours (4 wells per condition). After treatment, cells were spun down, and the supernatant from 2 wells was pooled to generate duplicate samples for each experiment and stored at −80°C until assayed by ELISA.

TNF-α and IL-10 in the supernatant was quantified using the human TNF-α ELISA Ready-SET-Go (eBioscience, San Diego, CA) and Human IL-10 ELISA MAX Standard Sets (Biolegend, San Diego, CA) following the manufacturer's instructions. Standards were diluted from 7.5–2000 pg mL^{-1} for TNF-α and 3.6–250 pg mL^{-1} for IL-10. Cell supernatant or media was diluted 5–80 fold to be measured within the standard curve range. After subtraction of media-only controls, the average values of triplicates were determined using the standard curve generated with known concentrations of human TNF-α or human IL-10.

LAIR-1 knockdown using siRNA

3×10^5 mBMDM cells per well were seeded on a 12-well cell culture treated plate 24 h before transfection. Transfection using Viromer BLUE transfection reagent (Lipocalyx, Germany) was performed according to the manufacturer's protocol. Briefly, Viromer BLUE was diluted (1:89 v/v) in the provided Buffer Blue, and the ON-TARGETplusSMARTpool mouse LAIR-1 siRNA or control nontargeting siRNA (both from GE Dharmacon) was also diluted independently to 2.5 μM in the Buffer Blue. 90 μL of diluted Viromer BLUE was added to 10 μL of diluted siRNA, and the mixture was incubated at room temperature for 15 min. For transfection, 100 μL of the Viromer BLUE-siRNA complex was added to each well of the 12-well plate by dispensing dropwise to 400 μL of serum-free media. Four hours later, the Viromer BLUE-siRNA containing media was replaced with fresh growth media. After 24–48 h of transfection, LAIR-1 knockdown efficiency was evaluated by flow cytometry using PE labeled LAIR-1 antibody (eBioscience).

Multiplexed cytokine assay

mBMDMs were cultured on LAIR-1 LP conjugated surfaces and cysteine-capped surfaces, followed by stimulation with 0.3 ng mL^{-1} LPS and 0.1 ng mL^{-1} IFN-γ. Cell culture supernatants were collected after 18 h of stimulation. Cytokine levels were measured with a Luminex 32-plex mouse cytokine/chemokine array (Eve Technologies, Canada). Results were analyzed in R and a heat map was generated after normalization to the mean of each analyte secreted from cysteine-capped surface with stimulation. All statistical analyses were also performed using R.

Statistical Analyses

Data presented are from at least three independent experiments ($n \geq 3$, with each independent experiment performed in duplicate or triplicate), and values are represented as the mean ± the standard error of the mean (S.E.M.), unless otherwise indicated. Data were analyzed using Student's two-sided *t*-test by either Excel or R, with $p < 0.05$ considered to be statistically significant. Any normalized data to remove technical variance was stated in each figure caption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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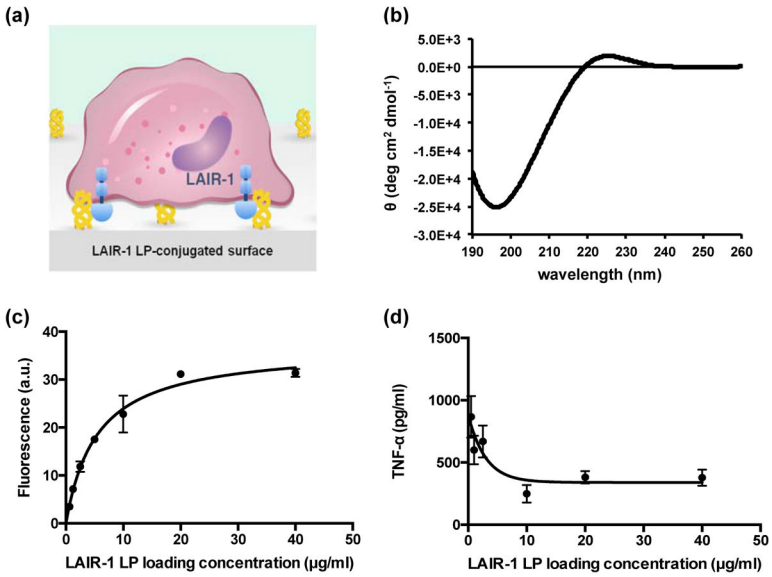

Figure 1.

Schematic and characterization of peptide (LAIR-1 LP). (a) LAIR-1 LP (depicted in yellow) conjugated on surface enables interaction with LAIR-1 (blue) expressed by cells. (b) Representative circular dichroism (CD) wavelength spectra for LAIR-1 LP. (c) Varying concentrations of fluorescently labeled LAIR-1 LP was incubated for conjugation, showing that surface saturation of peptide occurs at approximately 10–20 μg/mL LAIR-1 LP loading concentration. (n=2, each performed in triplicate.) (d) Secretion of TNF-α by mouse macrophages that were cultured on maleimide surfaces incubated with varying concentrations of LAIR-1 LP (0.1 μg/mL to 40 μg/mL). After washing unconjugated peptide, mBMDM cells were seeded and stimulated with LPS (0.3 ng/mL) and IFN-γ (0.1 ng/mL), and TNF-α secretion was measured after 18 h of stimulation. Data for mBMDM represent mean ± S.E.M., with n=3.

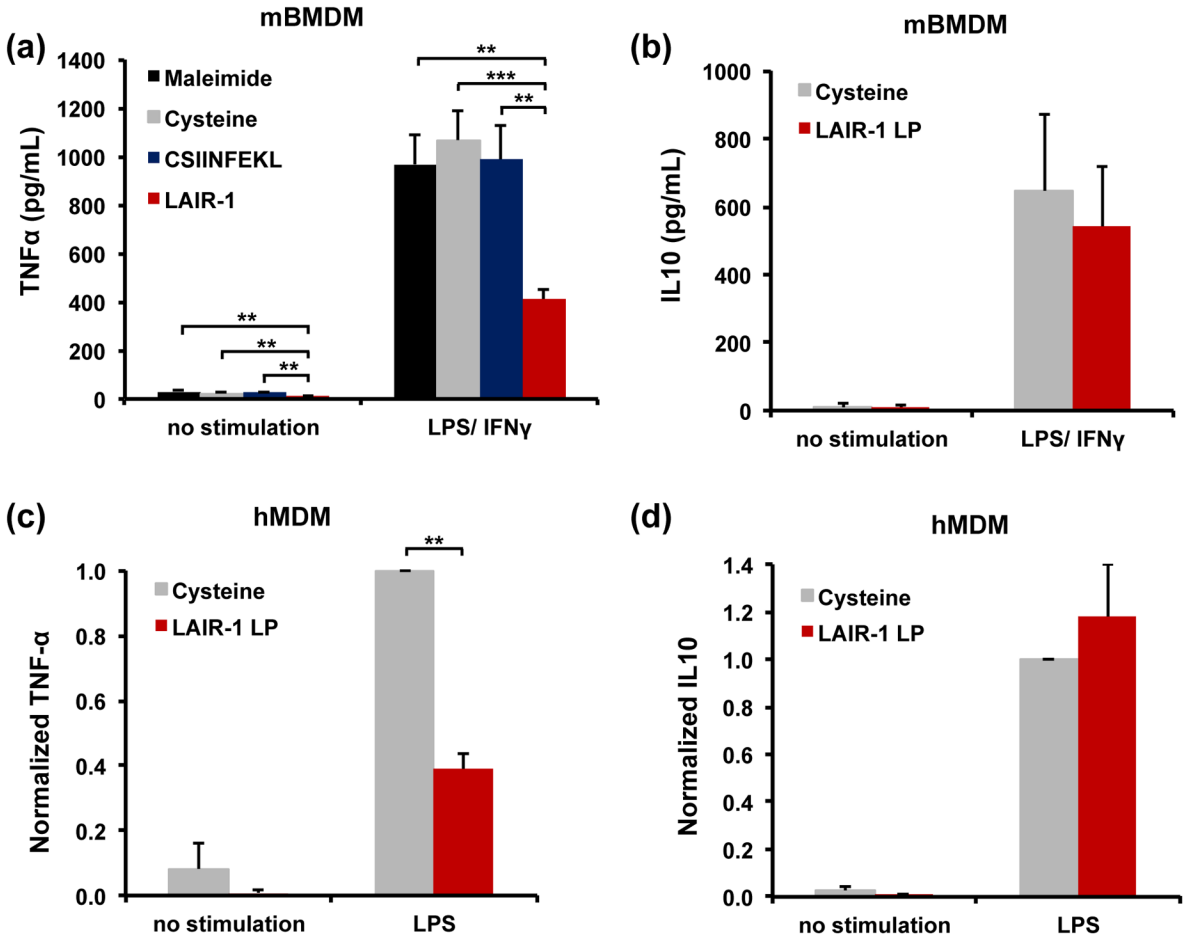

Figure 2.

In vitro inflammatory response of macrophages to LAIR-1 LP conjugated surfaces. (a) Secreted pro-inflammatory cytokine TNF-α and (b) anti-inflammatory cytokine IL-10 from mouse macrophages (mBMDM) cells seeded on LAIR-1 LP conjugated surfaces and on control surfaces (maleimide-activated, cysteine-capped, and CSIINFEKL-conjugated as a negative control peptide), followed by stimulation with LPS (0.3 ng/mL) and IFNγ (0.1 ng/mL) for 18 h. (c) Secreted pro-inflammatory cytokine TNF-α and (d) anti-inflammatory cytokine IL-10 from human macrophages (hMDM) cells seeded on LAIR-1 LP conjugated surfaces and on cysteine-capped control surfaces, followed by stimulation with LPS (10 ng/mL) for 19 h. Data for hMDM was normalized to mean of cysteine-capped surface with stimulation. All data represent mean ± S.E.M., across n ≥ 3 independent experiments. **$p < 0.01$, and ***$p < 0.001$ as determined by Student's *t*-test.

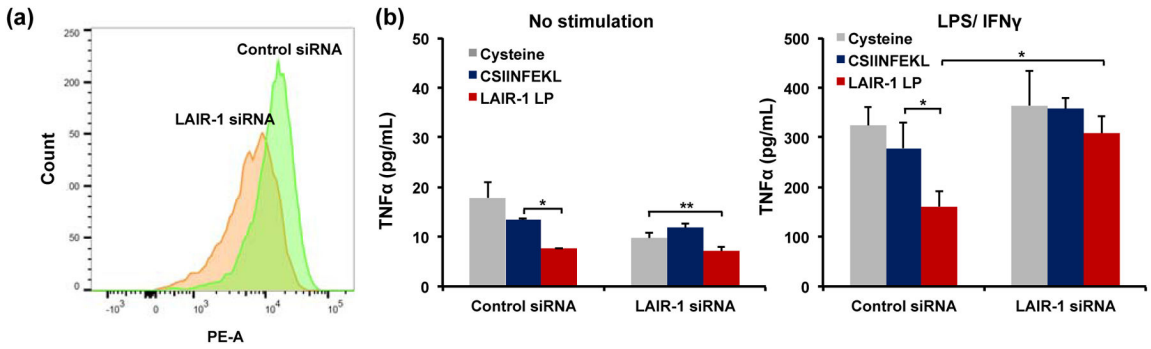

Figure 3.

Effect of LAIR-1 knockdown on mouse macrophage activation on LAIR-1 LP surfaces. (a) Expression of LAIR-1 on mouse macrophages (mBMDM) transfected with LAIR-1 or non-target control siRNA. (b) TNF-α secretion level from viable transfected mBMDM cells cultured on surfaces conjugated with cysteine, CSIINFEKL, and LAIR-1 LP (20 μg/mL as a loading concentration), with or without stimulation with LPS (0.3 ng/mL) and IFN-γ (0.1 ng/mL) for 18 h. Data represent mean + S.E.M. (n=3). *$p < 0.05$; **$p < 0.01$.

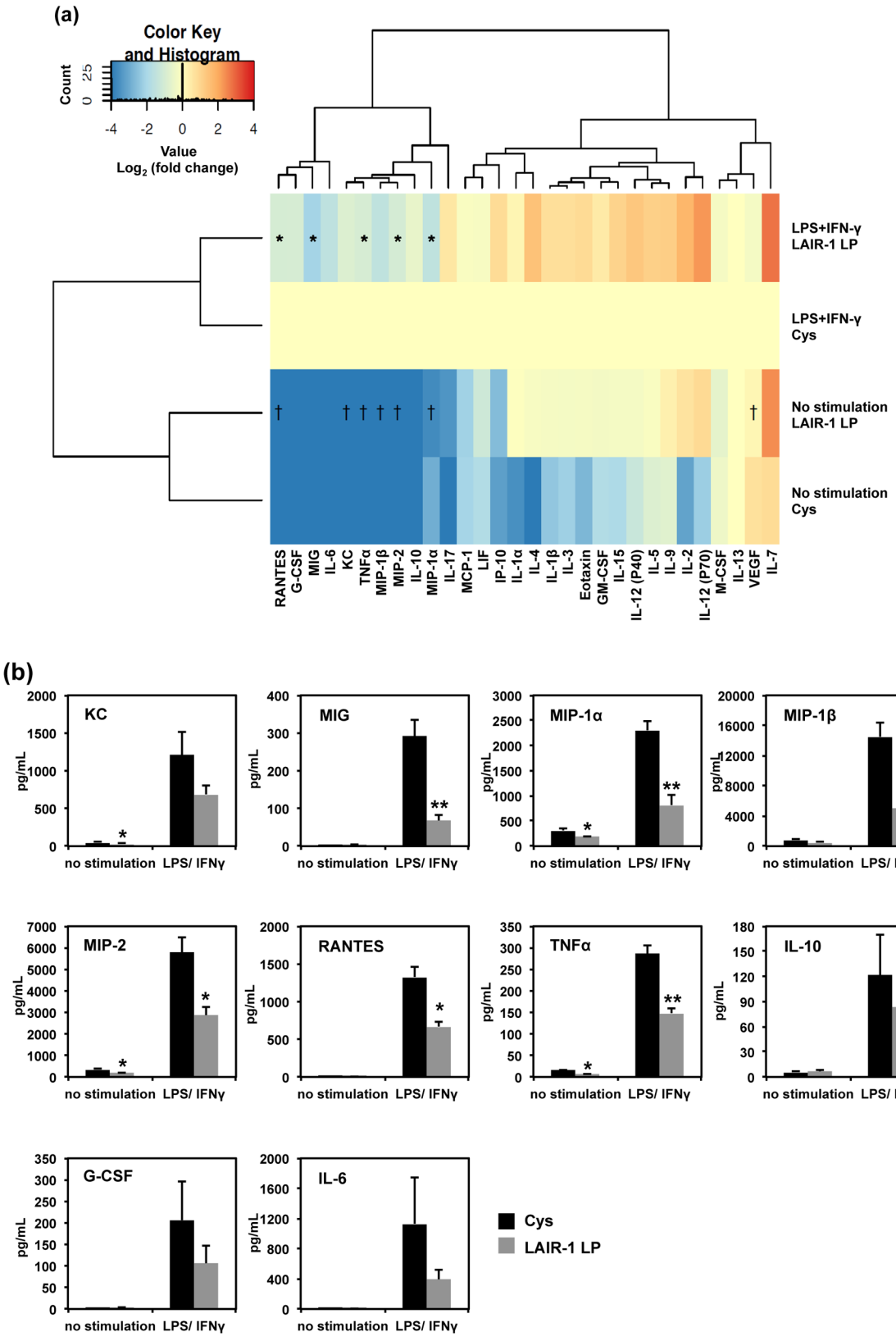

Figure 4.

Effect of LAIR-1 binding on mouse macrophage cytokine secretion. (a) Heat map depicting the fold change of cytokines/chemokines secreted by mBMDM after culture on LAIR-1 LP conjugated or cysteine-capped surface, with or without stimulation of 0.3 ng/mL LPS and 0.1 ng/mL IFN-γ for 18 h. Data were normalized to mean of cysteine-capped surface with stimulation. Values are Log_2 (fold change to Cys-capped surface with LPS+IFN-γ), with blue indicating lower levels, and red indicating higher levels compared to normalized value of 1.0. * and † denote $p < 0.05$ comparing LAIR-1 LP conjugated surface and cysteine-capped surface, with and without stimulation, respectively, by Student's *t*-test (n=3). (b) Bar graphs of selected cytokine/chemokine secretion, represented by mean + S.E.M. *$p < 0.05$ and **$p < 0.01$ comparing LAIR-1 LP conjugated surface and cysteine-capped surface, for no stimulation and LPS/IFN-γ stimulated groups, by Student's *t*-test (n=3). Abbreviations: KC (keratinocyte-derived chemokine, CXCL-1), MIG (monokine induced by gamma interferon, CXCL-9), MIP-1α (macrophage inflammatory protein, CCL-3), MIP-1β (CCL-4), MIP-2 (CXCL-2) and RANTES (regulated upon activation, normal T-cell expressed, and secreted, CCL-5).