MULTIVALENT STRUCTURE OF GALECTIN-1-NANOGOLD COMPLEX SERVES AS POTENTIAL THERAPEUTICS FOR RHEUMATOID ARTHRITIS BY ENHANCING RECEPTOR CLUSTERING

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Abstract

Cellular behaviour is controlled by numerous processes, including intracellular signalling pathways that are triggered by the binding of ligands with cell surface receptors. Multivalent ligands have multiple copies of a recognition element that binds to receptors and influences downstream signals. Nanoparticle-ligand complexes may form multivalent structures to crosslink receptors with high avidity and specificity. After conjugation of galectin-1 onto gold nanoparticles, the resulting nanogold-galectin-1 (Au-Gal1) bound with higher affinity to Jurkat cells to promote CD45 clustering and inhibition of its phosphatase activity, resulting in enhancement of apoptosis via caspase-dependent pathways. Au-Gal1 injected intra-articularly into rats with collagen-induced arthritis (CIA) promoted apoptosis of CD45, the major receptor for galectin-1 on T cells, acts as a negative or positive regulator of galectin-1-induced cell death and enhances phagocytic clearance of cells killed by galectin-1 (Walzel et al., 2000; Amano et al., 2003; Pang et al., 2009; Earl et al., 2010). Galectin-1 binds to the CD45 extracellular domain and reduces the tyrosine phosphatase activity of CD45 by receptor clustering (Walzel et al., 1999; Nguyen et al., 2001). The clustering of receptors

Keywords: Rheumatoid arthritis, ankle joint, apoptosis, cell-surface receptors, galectin-1, gold, inflammation, nanoparticles.

Introduction

Surface receptors play an important role in mediating the interface between a cell and its external environment. These molecules bind specific ligands to control numerous aspects of intracellular signalling (Shankaran et al., 2007). Multivalent ligands display multiple copies of recognition elements on the same scaffold, which allows for numerous ways in which receptors and ligands can interact (Gestwicki et al., 2002). Multivalent ligands bind multiple receptors and crosslink membrane receptors more efficiently to regulate the intensity and duration of intracellular signalling and subsequent responses. Nanoparticles coated with multiple ligands can act as multivalent ligands capable of crosslinking surface receptors (Jiang et al., 2008). Characterisation of nanoparticle platforms may provide a novel strategy to modulate cell signalling and develop therapeutic and diagnostic agents.

Galectin-glycoprotein lattice formation in T cells plays a critical role in regulating the threshold of signalling events, such as survival, activation and cytokine secretion (Rabinovich et al., 2007; Garner and Baum, 2008). Galectin-1 is a non-covalent homodimer consisting of subunits with a carbohydrate recognition domain responsible for β-galactoside binding in the reduced form, whereas the oxidised form containing intramolecular disulphide bonds is monomeric in structure and does not bind carbohydrates (Inagaki et al., 2000). Galectin-1 induces apoptosis of specific thymocyte subsets and activates T cells. The specific receptors involved in galectin-1-mediated T cell death are CD7, CD43 and CD45 (Camby et al., 2006). CD45 comprises up to 10 % of total cell surface proteins in T cells and regulates the signalling thresholds of receptors expressed on immune cells. The intracellular phosphatase domain of CD45 contributes to the majority of the tyrosine phosphatase activity in T cells and is critical for initiating T cell receptor-mediated signals (Hermiston et al., 2003; Trowbridge and Thomas, 1994).

CD45, the major receptor for galectin-1 on T cells, acts as a negative or positive regulator of galectin-1-induced cell death and enhances phagocytic clearance of cells killed by galectin-1 (Walzel et al., 1999; Fouillit et al., 2000; Nguyen et al., 2001; Amano et al., 2003; Pang et al., 2009; Earl et al., 2010). Galectin-1 binds to the CD45 extracellular domain and reduces the tyrosine phosphatase activity of CD45 by receptor clustering (Walzel et al., 1999; Nguyen et al., 2001). The clustering of receptors
is essential for galectin-1-mediated cell death of CD45-expressing T cells, although the mechanism of this process is unknown (Fouillit et al., 2000; Amano et al., 2003; Pang et al., 2009; Earl et al., 2010).

Rheumatoid arthritis (RA) causes chronic inflammation and joint destruction, and there is no effective therapy capable of improving disease symptoms and lessening joint destruction (Van der Heijde, 1995). The pro-inflammatory cytokines secreted from Th1 and Th17 T cells play an important role in the pathogenesis of RA. Galectin-1 can selectively bind Th1 and Th17 T cells due to differential glycosylation on their surface and induce cell death; it also acts to regulate cytokines by promoting Th2 cytokine production in Th2 cells (Toscano et al., 2007; Motran et al., 2008). However, the pro-inflammatory and therapeutic effects of recombinant galectin-1 protein or galectin-1 gene delivery on collagen-induced arthritis (CIA) in animal models that mimic human RA have been shown to be limited (Rabinovich et al., 1999; Wang et al., 2010). We have previously demonstrated similar pro-inflammatory activity by intra-articular administration of nanogold (Tsai et al., 2007). However, the observed pro-inflammatory effects were not significant enough for clinical use. Here, we developed nanogold-galectin-1 (Au-Gal1) complexes with multivalent structure to increase galectin-1 binding affinity to receptors and increase apoptotic signals by crosslinking CD45 on T cells. We expect that this multivalent property of Au-Gal1 complexes will have more therapeutic potential than free-form galectin-1 or nanogold for RA.

Materials and Methods

Protein adsorption
The protein concentration was required to stabilise the gold nanoparticles after 10 % NaCl was added to the nanoparticle solution. The protein amount was added to 1 mL of gold nanoparticle solution while stirring for 1 min. Following an additional 10 min of stirring, the solution was centrifuged at 12,000 g (at 4 °C) for 30 min to remove unbound excess proteins. The pellet was resuspended in 0.01 M sodium phosphate buffer (pH 7.4). The protein concentration in the supernatant was measured using a protein assay kit (Thermo Scientific Pierce, Rockford, IL, USA). The amount of protein bound to the surface of gold nanoparticles was calculated using the following formula: Protein bound to gold nanoparticles = Total protein added - Protein in the supernatant solution (Jiang et al., 2008).

Binding avidity
Jurkat cells were cultured in 96-well plates to 80 % confluency. Cells were fixed using 2 % paraformaldehyde and blocked with 1 % bovine serum albumin (BSA) for 30 min. Cells were incubated with varying concentrations of galectin-1 and Au-Gal1 for 1 h and then washed in phosphate-buffered saline (PBS) containing 0.1 % Tween 20 (PBST) to remove the unbound galectin-1 and Au-Gal1. Cells were treated with a biotinylated anti-galectin-1 antibody (R&D, Minneapolis, MN, USA) for 1 h, and then cells were washed in PBST. After streptavidin-conjugated horseradish peroxidase was added for 30 min, tetramethylbenzidine was added. Following a 20 min incubation, 2 N H₂SO₄ was added to stop the reaction. Absorbance was measured at 450 nm (Jiang et al., 2008).

Cell viability
Jurkat cells (1x10⁶) were plated in 96-well plates and incubated with different concentrations of galectin-1 or Au-Gal1 in serum-free RPMI medium. Following 6 h incubation, cells were incubated at 37 °C with 5 % CO₂. RPMI medium supplemented with 5 % foetal bovine serum (FBS) for 72 h. Cell viability was determined by trypan blue exclusion.

Western blot analysis
Jurkat cells (1x10⁶) were incubated under various conditions and washed three times with PBS. Cells were lysed with NP40 lysis buffer. Proteins were extracted by centrifugation and loaded onto a gel at a concentration of 20 μg per well. Total protein was resolved by SDS-PAGE (polyacrylamide gel electrophoresis) and transferred onto a nitrocellulose membrane. The protein involved in the galectin-1 signalling cascade, such as phosphorylated tyrosine (p-Tyr), caspase-8 and caspase-3, were detected using specific antibodies (Santa Cruz, Santa Cruz, CA, USA) and horseradish peroxidase-labelled goat anti-mouse IgG (Jackson, West Grove, PA, USA). The labelled membranes were incubated in an ECL working solution before imaging with the VersaDoc Model 5000 BioRad (Hercules, CA, USA) Imaging System.

CD45 clustering assay
For analysis of CD45 clustering, cells treated with galectin-1 or Au-Gal1 were fixed for 30 min with 2 % (wt/vol) paraformaldehyde and incubated for 1 h with a mouse monoclonal antibody to human CD45 (Santa Cruz) and biotin-labelled goat anti-mouse IgG. Cells showing CD45 aggregation were counted under a microscope.

Induction of CIA and delivery of Au-Gal1
To induce experimental arthritis of the ankle joint, male Sprague-Dawley rats at 8 weeks of age were immunised intradermally with 400 μg and 240 μg on days 0 and 8, respectively, of bovine type II collagen (Elastin Products, Owensville, MO, USA) emulsified in Freund’s incomplete adjuvant (Difco, Detroit, MI, USA) (Chen et al., 2011; Shiau et al., 2007). On day 14, they were injected intra-articularly with recombinant galectin-1 protein (40 μg), Au-Gal1 (containing 40 μg galectin-1), Au-BSA, Au or PB. There was 360 μg of nanogold in the Au-Gal1 and Au-BSA samples for treatment.

Clinical and radiographic assessment
The articular index was scored on a 0-4 scale (0 = no swelling or erythema, 1 = slight swelling and/or erythema, 2 = low to moderate oedema, 3 = pronounced oedema with limited joint usage, 4 = excess oedema with joint rigidity). Ankle circumference was determined using the following geometric formula: 2π(√(a²+b²/2)), where a is the laterolateral diameter and b is the anteroposterior diameter. The ankles were examined radiographically after the rats were sacrificed on day 24. Radiographs were scored on a
0-10 scale and were based on the width of the joint space, degree of bone destruction, and soft-tissue swelling (Wang et al., 2005).

Histopathologic and immunohistochemical assessment
Sections of paraffin-embedded ankle joints were prepared, stained with haematoxylin and eosin, and evaluated for the presence of synovial hyperplasia, cartilage erosion, and leukocyte infiltration. The sections were also stained with a polyclonal rabbit anti-von Willebrand factor antibody (Dako, Carpinteria, CA, USA) for microvessels, a mouse anti-rat monocyte/macrophage monoclonal antibody (ED1; Chemicon, Temecula, CA, USA) for infiltrating macrophages and a goat anti-rat CD4 monoclonal antibody (Shiau et al., 2007). Frozen sections of ankle joints were

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Fig. 1. Properties of Au-Gal1. (A) The stability of Au-Gal1 following addition of NaCl. (B) Spectroscopic detection of Au, Au-Gal1 and Au-BSA in NaCl solution. (C) TEM images of Au-Gal1. Scale bar = 20 nm. (D) Haemagglutination assay of galectin-1, Au-Gal1 and Au-BSA. Serial two-fold dilutions of galectin-1, Au-Gal1 or Au-BSA were mixed with 0.4% human O-type RBC suspension in U-shaped 96-well plates at room temperature for 30 min. RBC alone served as the negative control for haemagglutination.
also subjected to immunofluorescence staining with mouse anti-rat CD4 monoclonal antibody (BD PharMingen, San Diego, CA, USA) and Pand Alexa fluor594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA). Apoptotic cells were assessed with a DeadEnd colorimetric terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay kit (Promega, Madison, WI, USA).

Enzyme-linked immunosorbent assay (ELISA)
After induction of experimental arthritis, the ankle joints were injected intra-articularly with recombinant galectin-1 protein and Au-Gal1 in 50 μL of phosphate buffer (PB) on day 14. The levels of TNF-α, IFN-γ, IL-4, and IL-17 in the ankle homogenates of rats with CIA on day 16 were determined by ELISA (R&D, Minneapolis, MN, USA).

Statistical analysis
Data are expressed as mean ± SEM. Differences in rat articular index scores between two groups were examined by repeated-measures analysis of variance using SAS software, version 9.1 (GLM program; SAS Institute, Cary, NC, USA). Differences in the remaining data between two groups were compared by Student’s t-test. P values less than 0.05 were considered significant.

Results
Au-Gal1 complexes preserved the biological activity of galectin-1
To achieve the physical properties of a multivalent structure with nanoparticle-ligand complexes, a novel formulation of Au-Gal1 complexes in which recombinant human galectin-1 was conjugated onto a 13 nm nanogold surface was prepared. Au-Gal1 remained red in colour without an absorption spectrum shift when it was subjected to a 0.3 M NaCl solution, suggesting that the galectin-1 protein had good coverage on the particle surface to protect from particle aggregation in high-salt solution (Fig. 1A,B). Transmission electron microscopy (TEM) also demonstrated that Au-Gal1 had a uniform spherical structure with dispersed characteristics and was stable in the NaCl solution (Fig. 1C). The total size of the Au-Gal1 complexes was approximately 15 nm, which included the 13 nm gold nanoparticle core and the 1 nm layer of galectin-1 protein bound to the particle surface. On average, there were 100 mg of galectin-1 bound to 1 g of gold nanoparticles, which was equivalent to 66 galectin-1 molecules on a single 13 nm particle. To examine whether Au-Gal1 remained as biologically active as free galectin-1, a haemagglutination assay was performed. The lowest concentration required for Au-Gal1 to induce haemagglutination activity was as low as 0.039 μg/mL, whereas for free galectin-1, it was 160 μg/mL, indicating that Au-Gal1 exhibited a higher bioactivity than free galectin-1 (Fig. 1D).

Au-Gal1 enhanced T cell apoptosis via a caspase-dependent pathway
Galectin-1 is an important immune regulator that maintains T cell homeostasis by activating apoptosis through a caspase-dependent pathway (Brandt et al., 2008). We therefore evaluated whether Au-Gal1 was still able to induce apoptosis in T cells. Jurkat cells agglutinated upon treatment with either galectin-1 or Au-Gal1 but not upon mock or Au control treatments. This finding is in agreement with previous studies demonstrating that galectin-1 causes cell agglutination by crosslinking cell surface glycoconjugates (Lange et al., 2009). However, the interaction between Au-Gal1 and Jurkat cells was abolished by the addition of lactose, suggesting that Au-Gal1 bound Jurkat cells in a carbohydrate-dependent manner, that galectin-1 bound to the gold nanoparticle was in its reduced form and that the nanogold particle did not interfere with the interaction between galectin-1 and Jurkat cells (Fig. 2A). Furthermore, Au-Gal1 inhibited Jurkat cell proliferation after 72 h at an 8-fold lower concentration than that of galectin-1 (Fig. 2B), and this inhibition was attenuated by the addition of lactose (Fig. 2B). Addition of equal amounts of Au-BSA or nanogold alone had no effects on Jurkat cell proliferation (Fig. 2C). To further examine the reduction in cell growth, the induction of cell death by Au-Gal1 through caspase activation and DNA fragmentation were investigated. Full-length procaspase-8 p57 was cleaved to form caspase-8 p41/43 in Jurkat cells treated with Au-Gal1, whereas less caspase-8 activation was observed in cells treated with galectin-1, Au-BSA or Au. The procaspase-8 processing resulting from Au-Gal1 treatment was blocked by 40 mM lactose treatment (Fig. 2D). As a proteolytic signalling cascade is utilised by cells to activate caspases, we examined the effect of Au-Gal1 treatment on other caspases. As expected, a similar phenomenon was observed with procaspase-3; procaspase-3 p35 was processed to caspase-3 p20 after Au-Gal1 treatment, and this process was strongly attenuated in the presence of lactose (Fig. 2D). In addition, DNA fragmentation caused by cell apoptosis was only observed in Jurkat cells treated with Au-Gal1 (Fig. 2E). These results indicate that the induction of apoptosis in T cells by galectin-1 was augmented after galectin-1 was bound to gold nanoparticles.

Au-Gal1 increased binding affinity and CD45 clustering
The multivalent structure of Au-Gal1 may affect the binding avidity of galectin-1 to Jurkat cells. Therefore, the dissociation constant (Kd) values between Au-Gal1 and galectin-1 were compared. The Kd value of galectin-1 binding to Jurkat T cells was 22.81x10^-11 M, whereas it was 2.39x10^-10 M for Au-Gal1 binding to the same cells (Fig. 3A). The 10-fold stronger affinity of Au-Gal1 for Jurkat cells demonstrated by Kd may suggest prolonged receptor binding. We therefore evaluated whether clusters formed directly after Au-Gal1 binding. The localisation of CD45 on the cell surface was examined by confocal microscopy, and the percentage of cells with clustered CD45 molecules was calculated. Treatment of Jurkat T cells with Au-Gal1 resulted in an approximately 4-fold increase in CD45 molecules when compared with Jurkat cells treated with free galectin-1 (Fig. 3B,C). CD45 clustering on cells after galectin-1 binding has been suggested to facilitate auto-inhibition of intracellular protein tyrosine phosphatase.
Fig. 2. Au-Gal1 induced apoptosis in Jurkat cells. (A) Cells (2×10^6/well) cultured in 6-well plates were treated with galectin-1 (10 μg) with or without the concomitant addition of lactose (40 mM), or with 5 nM particle concentration of Au-Gal1, Au-BSA or Au for 30 min. Cells were examined for agglutination with an inverted phase microscope. Scale bar = 100 μm. (B) Cells (1×10^5/well) cultured in 96-well plates were treated with various concentrations of galectin-1 or Au-Gal1 for 6 h at 37 °C. They were further incubated in RPMI medium supplemented with 5 % FBS for 72 h. Cell viability was determined by trypan blue exclusion. (C) Cells (1×10^5/well) cultured in 96-well plates were treated with Au-Gal1 (10 μg/mL) in the presence or absence of lactose (40 mM), or with 5 nM particle concentration of Au-BSA or Au for 6 h at 37 °C. They were further incubated in RPMI medium supplemented with 5 % FBS for 72 h, and their viability was determined by trypan blue exclusion. (D) Cells (1×10^5) treated as described in (c) were subjected to immunoblot analysis for caspase-8 after 8 h and for caspase-3 after 10 h. (E) Cells (2×10^4/mL) treated as described in (c) for 12 h. DNA extracted from the treated cells were separated by electrophoresis in 1.5 % agarose gels for detection of DNA ladder formation.
Fig. 3. Binding affinity of Au-Gal1 influenced CD45 clustering and signalling. (A) Binding avidity analysis: dissociation constant ($K_d$) values for galectin-1 and Au-Gal1. (B) Laser confocal microscopy of cells incubated with galectin-1 or Au-Gal1. Cells were fixed and stained with a mouse monoclonal anti-human CD45 antibody and biotin-labelled goat anti-mouse IgG. (C) Percentage of CD45 clustering was calculated as follows 100×(CD45 clustering cells/total cells). Cells showing CD45 clustering were counted in three fields for each group. Data represent the mean ± SEM of four independent experiments. (D) Au-Gal1 induced tyrosine phosphorylation in Jurkat cells. Jurkat cells were stimulated with 10 μg/mL of Au-Gal1 at 37 °C. (E) Cells were stimulated with galectin-1 or Au-Gal1 for 2 h at 37 °C. The samples were then analysed by Western blotting with a monoclonal antibody against phosphotyrosine.
Galectin-1-nanogold complex enhances receptor clustering

Fig. 4. Au-Gal1 treatment increased CD4+ T cells undergoing apoptosis and reduced inflammatory cytokines in the ankle joints of rats with CIA. (A) Detection of CD4 and TUNEL double-positive cells. Rats were immunised with collagen on days 0 and 8 for induction of CIA. They received different treatments on day 14. Frozen sections of ankle joints collected on day 16 were subjected to immunofluorescence staining with mouse anti-rat CD4 monoclonal antibody and Alexa fluor594 goat anti-mouse IgG. Apoptotic cells were stained with TUNEL. More apoptotic CD4+ T cells were detected by TUNEL staining in Au-Gal1-treated synovial tissues. In the merged images, the right panels are higher-magnification views (original magnification ×2,000) of the respective boxed areas in the left (original magnification 400×, scale bar = 20 μm). (B) Number of CD4 and TUNEL double-positive cells in three randomly selected high-power fields (original magnification ×400) in each section. (C) Levels of IFN-γ, IL-4, IL-17 and TNF-α in the ankle homogenates of rats with CIA on day 16 were quantified by ELISA. Bars show the mean ± SEM from 6 samples per treatment group for b and c.
Fig. 5. Au-Gal1 treatment ameliorated clinical symptoms in rats with CIA. Rats were immunised with collagen on days 0 and 8 for induction of CIA. They received different treatments on day 14. (A) Reduction in the articular index score in the rats treated with Au-Gal1. (B) Reduction in the ankle circumference in the rats treated with Au-Gal1. Bars in a and b show the mean ± SEM in 12 feet per group. (C) Representative photographs of the rat ankles on day 24. The results of plain radiography revealed that Au-Gal1-treated rats had more intact joint structure as compared with their control counterparts. Au-Gal1 reduced the radiologic scores on day 24 in rats. Bars show the mean ± SEM for 6 rats per group. (D) Representative histologic score and haematoxylin and eosin-stained joint sections on day 24. Results are the mean ± SEM for 6 rats per group.
activity, leading to enhanced galectin-1-induced cell death (Walzel et al., 1999). Fig. 3D demonstrates that cellular tyrosine phosphorylation was increased after treatment with Au-Gal1 in a time-dependent manner, and it was significantly higher in cells treated with Au-Gal1 compared with the other treatments. However, enhancement of tyrosine phosphorylation was abolished in the presence of lactose (Fig. 3E). Taken together, these results indicate that the multivalent structure of Au-Gal1 enhanced CD45 clustering, which led to modulation of membrane receptor signalling and alterations in cell behaviour.

Au-Gal1 enhanced CD4+ T cell apoptosis and ameliorated clinical symptoms of CIA

To further investigate the role of Au-Gal1 in promoting the apoptosis of CD4+ T cells in vivo as well as evaluate its therapeutic feasibility, galectin-1, Au-Gal1, Au-BSA or nanogold were administered intra-articularly to rats with CIA. Tissues from arthritic joints after 48 h of treatment were examined for apoptosis by TUNEL assay and for CD4+ T cells by immunohistochemical staining (Fig. 4A). A three-fold increase in apoptotic CD4+ T cells and lower numbers of CD4+ T cells were observed in the synovial tissue of animals treated with Au-Gal1, compared to those treated with galectin-1 only (Fig. 4B). As shown in Fig. 4C, the levels of the Th1 cytokine interferon-γ (IFN-γ), the Th17 cytokine interleukin (IL)-17 and the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) in the joint extracts were reduced by 50 % in the Au-Gal1-treated group compared with the Gal-1-treated group. However, the levels of IL-4 were not significantly altered in the Au-Gal1-treated group compared to the remaining groups (Fig. 4C). To further evaluate the therapeutic effects of Au-Gal1 on CIA, rats immunised twice with collagen were injected intra-articularly with Au-Gal1, PB, galectin-1, Au-BSA or nanogold after the onset of CIA. Both ankle circumference (Fig. 5A) and articular index score (Fig. 5B) were significantly smaller in the Au-Gal1-treated group than in the two control groups. We next assessed the radiographic and histologic changes in the ankle joints after the animals were sacrificed on day 24. Joint space narrowing and bone erosion were observed in the ankles treated with galectin-1, Au, Au-BSA or PB but were markedly attenuated in the Au-Gal1-treated ankles. The radiologic score, which was based on the width of the joint space, degree of bone destruction and soft-tissue swelling, was also significantly lower in the Au-Gal1-treated group than in the control groups (Fig. 5C). To confirm the improvement of clinical symptoms, histological examination of the joint tissues was conducted. Histopathologic analysis of galectin-1-, Au-, Au-BSA- or PB-treated joint tissues revealed synovial hyperplasia and obvious bone erosion. However, the joint tissues from Au-Gal1-treated animals showed relatively intact cartilage on the joint surface and slight hyperplasia of the synovial membrane. Furthermore, the histologic score was significantly lower in the Au-Gal1-treated group than in the other groups (Fig. 5D). Collectively, these findings indicate that treatment with Au-Gal1 reduced the severity of arthritic symptoms in rats with CIA, as determined by examination of the clinical, radiographic and histologic features of their ankle joints. Immunohistochemical staining also demonstrated that fewer infiltrating CD4+ T cells, macrophages and microvessels were present in the synovium of the Au-Gal1-treated group compared to the control groups (Fig. 6A, B, C). These results suggest that the beneficial effect of Au-Gal1 on arthritic symptoms may be attributed to its cytotoxic effect on infiltrating CD4+ T cells in arthritic tissues. Taken together, our data showing that Au-Gal1 reduced the severity of CIA as indicated by the clinical, radiographic and histologic features suggest that Au-Gal1 nanoparticles act as a multivalent form of galectin-1 and may be more effective than other molecules in protecting joint integrity.

Discussion

Nanoparticles have been engineered into biosensors, molecular scale fluorescent tags, imaging agents, targeted molecular delivery vehicles and other useful biological tools with chemically modifiable surfaces onto which various ligands can attach (Liu et al., 2007). The multivalent enhancement of the KD is a key factor in improving biological targeting by drug delivery platforms (Hong et al., 2007). Multivalent nanoparticles coated with targeting peptides have been studied in image and therapeutic systems (Huang et al., 2009; Shokeen et al., 2011). In this work, we investigated a new application of nanoparticles to reveal the multivalent effects of biological molecules. When galectin-1 was conjugated to nanoparticles, the resulting complex, Au-Gal1, led to increased binding affinity to target cells and enhancement of T cell apoptosis by the clustering of CD45 molecules. Clustered CD45 molecules promoted apoptosis via caspase-dependent pathways. Nanoparticles therefore not only play a role in vector delivery to increase payload influx or imaging detection, but they can also be conjugated to ligands to regulate receptor distribution and downstream cell signalling.

In CIA, Au-Gal1 enhanced CD4+ T cell apoptosis and reduced the production of Th1 and Th17 cytokines, as well as TNF-α in the synovium. Here, low doses of Au-Gal1 showed better therapeutic effects than free galectin-1. In our previous study, administration of gold nanoparticles in the early stages of CIA reduced disease progression by inhibiting vascular endothelial growth factor (VEGF) activity, but this strategy proved less effective at reducing the severity of arthritis when administered after the onset of the disease. In the current study, the clinical, radiographic and histologic features in rats were reduced by Au-Gal1 administration after the onset of disease. Thus, in the later stages of disease, therapeutic targeting of T cells was more effective than targeting VEGF in reducing the severity of the disease in the joint.

In conclusion, our study provides a novel approach for the application of nanoparticles, because the multivalent property of the nanostructure actively regulates membrane receptor organisation to affect cell signalling. This concept will have a significant impact on the design of nanodevices with great potential for therapeutic applications.
Fig 6. Au-Gal1 treatment reduced infiltrating CD4+ T cells and macrophages as well as microvessel density in the synovium of rats with CIA. Rats were immunised with collagen on days 0 and 8 for induction of CIA. They received different treatments on day 14. Paw joints were harvested on day 24 and the joint tissues were processed for analysis of CD4+ T cells (A), macrophages (B), and microvessels (C) by immunohistochemical staining. Scale bars = 100 μm.
Acknowledgements

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References


Discussion with Reviewer

C. de Bari: Did the authors detect any side effects after intra-articular administration of Au-Gal1?

Authors: During the 24 day observation period, rats treated intra-articularly with Au-Gal1 did not show noticeable adverse effects as they looked healthy and vigorous. However, long-term studies are required to examine the potential toxicity of Au-Gal1.

C. de Bari: Rheumatoid arthritis tends to be polyarticular, hence requiring systemic treatment. Do the authors envisage a systemic route of administration of the Au-Gal1? If so, which one? What would the expected side effects be?

Authors: Because RA is a systemic disease that commonly involves multiple joints and several organs, systemic administration may be more suitable for the clinical setting. However, since T cells are the main target of Au-Gal1, the authors cannot exclude the possibility that systemic delivery of Au-Gal1 might also induce apoptosis and hence reduce the number of circulating T cells. Using the intra-articular route of administration, Nagata and colleagues (Nagata et al., 2009, additional reference) showed that injection of double-stranded microRNA may move from the joint cavity into the systemic circulation as higher levels of microRNA-15a were detected in the liver, suggesting that the effect of intra-articular administration is not restricted to the injected joints. In this regard, whether intra-articular injection of Au-Gal1 can gain access into the systemic circulation deserves further experimental investigation. It would also be of interest to compare intra-articular and systemic routes of Au-Gal1 administration in animal models of arthritis in terms of therapeutic effects and potential side effects.

C. de Bari: Where would the authors position the Au-Gal1 in the current treatment algorithm of inflammatory arthritis? Do they see potential for a specific clinical niche?

Authors: The present study provides a proof-of-concept for the use of Au-Gal1 as a novel therapeutic agent for the treatment of RA. Compared to our previous study (Tsai et al., 2007, text reference) showing that nanogold alone can reduce arthritis symptoms in the rat CIA model when administered before, but not after the onset of the disease due to its anti-angiogenic activity, Au-Gal1 is more effective as a therapeutic agent. The results presented in this study suggest that T cells may serve as a therapeutic target for RA even after neovascularisation has been established in the early stages of arthritis. Au-Gal1 may have therapeutic effects in the late stages of arthritis to prevent disease progression. Biologically-based immunotherapies, such as TNF-α antagonists, have been marketed for treating RA and have improved the life quality of many patients. However, a substantial number of patients have an inadequate or unsustained response. Au-Gal1 may have its niche to serve as a T cell-targeted therapeutic agent for RA.

C. de Bari: What would be the advantages of intra-articular Au-Gal1 over intra-articular corticosteroids?

Authors: Intra-articular injection of corticosteroids is used for the relief of joint pain and swelling through their anti-inflammatory activity. If the results from the animal study can be extrapolated to humans, we presume that the advantages of intra-articular Au-Gal1 over intra-articular corticosteroids would be that common and irreversible side effects of corticosteroids, such as osteoporosis, may not be expected to occur following Au-Gal1 treatment. Our previous report showed that nanogold can bind directly to VEGF in human RA synovial fluid and inhibit its bioactivity (Tsai et al., 2007), suggesting that Au-Gal1 may also exert anti-angiogenic effect when nanogold is dissociated from the Au-Gal1 complex.

Additional Reference