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Galectin 1 suppression of collagen-induced arthritis

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Keywords

Apoptosis, collagen-induced arthritis, galectin-1, gene therapy, T cells

Context

Galectin 1 (GAL-1) is a ?-galactosidase-binding protein which participates in carbohydrate recognition processes such as those involved in cell adhesion, cell growth regulation and metastasis. In addition GAL-1 induces apoptosis of activated mature T cells and subsets of immature thymocytes through engagement of CD43 or CD45. These observations suggest that GAL-1 may participate in the induction of immune tolerance. The murine DBA/1 collagen-induced arthritis (CIA) model was used to compare the therapeutic effect of GAL-1 delivery daily as protein or constitutively from genetically modified syngeneic cells. This study was performed to examine the therapeutic potential of GAL-1 in the CIA model, and to examine the contribution of T cell apoptosis to GAL-1 action.

Significant findings

Permanently transfected syngeneic fibroblasts used in gene therapy were shown by western blot to express both cell-associated and secreted mGAL-1.

The *in vitro* antigen presentation assay demonstrated GAL-1 inhibition of IL-2 secretion. Transfected DBA/1 fibroblasts displayed increased inhibition of IL-2 with increase in cell number, and with rhGAL-1 the effect was shown to be dose- and time-dependent. In the CIA model both rhGAL-1 and genetically delivered mGAL-1 significantly inhibited the development of CIA as assessed by paw swelling, clinical score and number of paws affected. The clinical findings were supported by histological examination, which confirmed that the paws of GAL-1 treated mice were only mildly arthritic compared to controls. Gene therapy and protein therapy with GAL-1 also significantly reduced the serum total anti-CII immunoglobulin level, and both treatments reduced anti-CII IgG2a levels and increased anti-CII IgG1 levels compared to those in control serum. Gene therapy also altered the cytokine secretion profile of lymph node cells cultured with CII for 72 h with an increase in IL-5 and a decrease in IFN-? observed compared to the control group. Lymph node cells from treated animals were more sensitive to CII-induced apoptosis, as determined by hypodiploid DNA content and DNA fragmentation after 24 h culture compared to lymph node cells obtained from control animals.

Interestingly, antigen-induced cytokine secretion and apoptosis of spleen cells was not altered by mGAL-1 treatment.

Comments

This innovative study employed GAL-1, a novel therapeutic agent, in the treatment of murine CIA. The therapeutic effects observed are outstanding, with disease progression arrested soon after commencing treatment by protein or gene therapy. The therapeutic effect of GAL-1 was attributed to its ability to induce apoptosis of activated T cells, resulting in a switch from Th1 pro-inflammatory to a Th2 response. *In vitro* studies with HCQ6s provide further support of this apoptotic effect and add weight to the proposed mechanism of action. Fas ligand gene transfer has previously been shown to ameliorate CIA through apoptotic activity however, Fas ligand induces the apoptosis of all cells expressing high levels of the death factor Fas, and does not selectively target activated T cells.

The authors note that constitutive expression of GAL-1 by gene therapy would clearly be detrimental but, combined with the ability to regulate GAL-1 expression, it would represent a powerful therapeutic approach. The proposal that GAL-1 mediated apoptosis of activated arthritogenic T lymphocytes could reset the immune system and induce tolerance to autoantigens driving disease is intriguing. Indeed, such a therapeutic effect would have potential applications beyond arthritis and autoimmunity.

Methods

Murine GAL-1 (mGAL-1) DNA was cloned into the eukaryotic expression vector pCDNA3 and transfected into conditionally immortalised DBA/1 embryonic fibroblasts. Selected clones were pooled and GAL-1 expression confirmed by western blot. Recombinant human GAL-1 (rhGAL-1) was expressed from the vector pH14GAL in *Eschericia coli*strains SCSI and Y1090 and purified by affinity chromatography.

Subsequent experiments were designed to examine the effect of GAL-1 in an *in vitro* antigen presentation assay and an *in vivo* antigen-driven arthritis model. In the *in vitro* assay collagen II (CII) was presented by DBA/1 spleen cells to a CII-specific T cell hybridoma (HCQ6), and interleukin-2 (IL-2) production was measured by enzyme-linked immunosorbent assay (ELISA). The influence of GAL-1 on antigen presentation was assessed by including GAL-1 transfected DBA/1 fibroblasts or rhGAL-1 in the assay. Arthritis was induced in male DBA/1 mice by immunisation with bovine CII emulsified in complete Freunds adjuvant. Arthritis developed in these animals between 20 and 23 days after immunisation. Gene therapy with GAL-1 was performed by intraperitoneal injection of 4×10^6 engineered DBA/1 fibroblasts on the day of disease onset, whilst control animals received fibroblasts transfected with pCDNA3. Animals treated with rhGAL-1 received daily intraperitoneal injections for 11 days from the day of disease onset and control animals received daily injections of phosphate buffered saline (PBS). Arthritis development was monitored daily and both paw thickness and clinical

score recorded. The experiment was terminated 12 days after disease onset, at which point serum was collected for immunoglobulin measurement by ELISA. Arthritic hind paws were removed for histopathological assessment of disease. Cells derived from inguinal lymph nodes and spleens were cultured and assessed for CII-induced cytokine production, apoptosis and DNA fragmentation.

References

1. Rabinovich GA, Daly G, Dreja H, Tailor H, Riera CM, Hirabayashi J, Chernajovsky Y: Recombinant galectin-1 and its genetic delivery suppress collagen-induced arthritis via T cell apoptosis. J Exp Med. 1999, 190: 385-397.