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Galectin-7 in Cardiac Allografts in Mice: Increased Expression Compared With Isografts and Localization in Infiltrating Lymphocytes and Vascular Endothelial Cells

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ABSTRACT

We sought to identify elevated expression of galectin-7, a T-cell–binding protein, in renal allograft recipients undergoing acute rejection episodes. Allografts and isografts were examined immunohistologically and using quantitative Western blot analysis for galectin-7 protein. The expression of galectin-7 in T lymphocytes from draining lymph nodes in the recipient mice was examined using flow cytometry. We observed galectin-7 to gradually increase with time in the allografts to be significantly higher than that in isografts at days 3, 5, and 7 postoperative: 0.85 ± 0.03 versus 0.69 ± 0.05; 1.15 ± 0.11 versus 0.81 ± 0.02; and 2.02 ± 0.12 versus 0.81 ± 0.05 ($P < .05$). The majority of galectin-7 was located in the infiltrating lymphocytes and vascular endothelial cells. Furthermore, the percentage of CD4+galectin-7+ and CD8+galectin-7+ T cells from draining lymph nodes in the allograft group was higher than that in the isograft group: 28.0 ± 1.0% versus 1.2 ± 0.2%; and 12.4 ± 0.8% versus 0.4 ± 0.1% ($P < .01$). In conclusion, galectin-7 relates to acute allograft rejection and T-cell responses possibly as an accelerant.

GALECTINS, a family of conserved β-galactoside–binding proteins, have received increasing attention as novel regulators of immune cell apoptosis, proliferation, and actions.[1–4] Recent evidence has shown that galectins play roles in allograft rejection by controlling T-cell homeostasis and activation. Among 3 members of the galectin family, galectin-1 modulates Th1 cell and Th17 cell growth and apoptosis by resulting in an evident redistribution of glycoproteins on the cell surface.[5] Galectin-8 provides costimulatory signals to induce proliferation of naive CD4+ T cells in mice.[6] Galectin-9, like galectin-1, selectively induces Th1 cell death through interaction with T cell immunoglobulin and mucin domain 3 (Tim-3), a cell-surface protein expressed specifically on Th1 but not Th2 cells.[7] Our previous studies have revealed that galectin-9 inhibits T-cell activation, therefore, significantly prolonging allograft survival.[8,9]

Galectin-7, a member of this protein family that is expressed preferentially in stratified epithelia within various types of cancer is associated with apoptosis and proliferation.[10–13] It can be up-regulated significantly in the process of wound healing.[13] Recent studies have indicated that galectin-7 may be related to immune responses in transplantation, as a T-cell–binding protein whose binding ability is increased upon activation. Rossi et al.[14] have shown that the binding sites are sugar chains of CD3 surface molecules on T lymphocytes. T-cell proliferation and differentiation is known to be regulated by complex N-glycans.[15] We have also identified increased expression of galectin-7 in serum from renal allograft recipients compared with normal volunteers,[16] raising the possibility that galectin-7 is a mediator in acute allograft rejection.

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To test the hypothesis that galectin-7 correlates with allorejection and to examine the localization of galectin-7 in grafts, we examined acute murine cardiac transplantation rejection in the grafts using immunohistochemistry and among the T cells from draining lymph nodes using flow cytometry.

MATERIALS AND METHODS

Mice

Male BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were bred separately under controlled condition at our Animal Facility. All experimental procedures were performed under a protocol approved by our Institutional Animal Care and Use Committee.

Heterotopic Cardiac Transplantation

Mouse abdominal cardiac transplantation was performed as previously reported:[17] mice (body weight, 20–25 g) were anesthetized with intraperitoneal pentobarbital. The hearts of BALB/c (H-2^d) donors were placed in the abdomen of the recipients. Nine allografts (BALB/c→BALB/c) and 9 isografts (BALB/c→C57BL/6) were performed. Graft function was evaluated by daily palpation of the abdomen of the recipients. Three mice in each group were humanely killed at 3, 5, and 7 days after transplantation. The hearts were removed quickly, cut into 2 pieces, and stored in a −80°C refrigerator. The abdominal draining lymph nodes harvested postoperatively at day 7 were immediately analyzed using flow cytometry.

Protein Extraction and Western Blot Analysis

Eighteen corresponding native hearts from recipient mice were used as controls.

The homogenized heart tissues were centrifuged for 20 minutes at 13,000 rpm/4°C. The supernatant proteins were quantified using the Bradford assay before separation on 12% sodium dodecyl sulfate–polyacrylamide gels and transfer onto polyvinylidene difluoride membranes. After transferring to the membranes, they were blocked with 1× TBS-T (Tris-buffered saline with Tween-20) containing 10% nonfat dry milk for 1.5 hours, incubated with 1:200 primary antibody against galectin-7 (rat anti-mouse, R&D, Minneapolis, MN, USA) overnight at 4°C, and incubated with 1:1000 horseradish peroxidase-conjugated goat anti-rat antibody (ZSGB, China). GAPDH served as a loading control. The images were visualized using an enhanced chemiluminescence detection kit (Beyotime, Shanghai, China).

Histology and Immunohistochemistry

The cardiac grafts fixed in 10% formalin were embedded in paraffin. Approximately 4-μm thick sliced sections were stained with hematoxylin and eosin. Parallel sections were analyzed for galectin-7 using immunohistochemistry. The primary antibody against galectin-7 was applied overnight at 4°C at a 1:50 dilution in 1% bovine serum albumin, followed by goat anti-rat bridging antibody (1:100 in phosphate-buffered saline, 20 minutes at room temperature; ZSGB, Beijing, China). Finally, the sections were stained with 3,3′-diaminobenzidine for 2 minutes and redyed with hematoxylin. We also prepared negative control without the primary antibody.

Flow Cytometric Analysis

The single-cell suspensions from the abdominal draining lymph nodes of the recipients were harvested by grinding before filtration through a 200 mesh sieve. After washing twice, phycoerythrin-conjugated anti-CD4 (eBioscience) and allophycocyanin-conjugated anti-CD8 (eBioscience) were simultaneously added to the cell suspensions for 30 minutes at 4°C in the dark. For intracellular staining, the fixed cells were permeabilized by a ready-made buffer (eBioscience, San Diego, Calif, USA) before staining with primary anti–galectin-7 antibody overnight at 4°C in the dark, followed by a fluorescein isothiocyanate-conjugated anti-rat antibody for 30 minutes at room temperature in the dark (ZSGB). Finally, the pellets resuspended in 200 μL of staining buffer were analyzed using a FACScan flow cytometer (Becton Dickinson) with FACS Aria software (BD Biosciences, San Jose, Calif, USA). The single-cell suspensions from the abdominal draining lymph nodes of normal C57BL/6 mice were used as negative controls.

Statistical Analysis

The results were expressed as mean values ± standard error of the mean (SEM). An independent-sample student's t test was used to analyze differences between the 2 groups. Multiple groups were compared by one-way analysis of variance (ANOVA). Differences were deemed statistically significant if $P < .05$.

RESULTS

Acute Rejection Progressively Worsened in the Allografts

The histological results of the isografts and allografts harvested at each time point are presented in Figure 1. All cardiac grafts revealed evidences of inflammatory responses. Notably, the isografts showed mild changes with slight cellular infiltration, little or no edema, no hemorrhage and no necrosis. There was no remarkable increase in severity between days 3 and 7. The histological changes in the allografts were characteristic of an, alloresponse, which gradually worsened until the time of acute rejection at 7

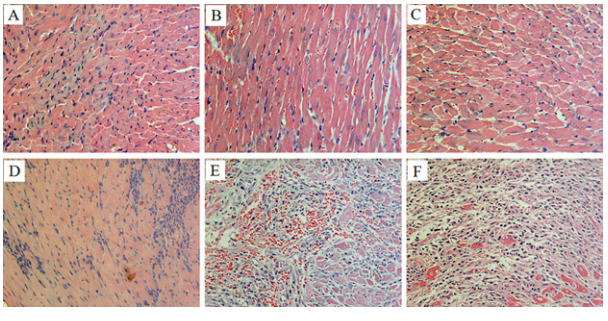

Fig 1. Representative histological examination of cardiac grafts at each time point (original magnification ×400). Isografts **(A)**, **(B)**, and **(C)** show the histological results at days 3, 5, and 7 posttransplantation, respectively. They exhibited mild changes with little cellular infiltrate, little or no edema, no hemorrhage, and no myocardial necrosis. Allografts **(D)**, **(E)**, and **(F)** show the histological results at days 3, 5, and 7 after transplantation, respectively. **(D)** The photomicrograph shows severe myocardial edema and mild lymphocytic infiltrate on day 3. **(E)** Extensive areas of hemorrhage and moderate lymphocytic infiltrates were observed on day 5. **(F)** Large areas of myocardial necrosis and pronounced lymphocytic infiltrates appeared on day 7. The results are representative of 3 experiments.