FIBROBLASTS TO COLLAGEN INTERACTIONS IN THE PROCESS OF COLLAGEN GEL CONTRACTION: ANALYSIS OF THE MECHANISM OF COLLAGEN GEL CONTRACTION USING MONOCLONAL ANTIBODIES AND LECTINS

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The culture of fibroblasts in three-dimensional collagen gels (collagen gel culture) has been shown to provide a more physiological environment to the cells than a conventional monolayer culture on plastic [1]. In the collagen gel culture of fibroblasts, the cells bind collagen fibrils, which results in marked contraction of the gels [2]. This contraction is an interesting phenomenon as a model for studying cell to collagen interactions, because it has been shown that fibroblasts remodel and reorganize collagen fibrils into dermal-like tissue during this process [3-4]. In the present study, we have applied a variety of techniques to elucidate the mechanism of contraction of collagen gels by human dermal fibroblasts.

One of the most successful trials was to have obtained monoclonal antibodies (mAbs) which inhibit the contraction of collagen gels. MAbs were prepared against fibroblast surface antigens. Of 98 clones obtained 2 clones, mAb A3A5 and B1A4, were found to be effective in suppressing the fibroblast-mediated collagen gel contraction. When fibroblasts were cultured in collagen gels, the cells spread three-dimensionally prior to contraction of gels. However, in the presence of culture supernatants of A3A5 and B1A4, most fibroblasts in the gel remained round in shape. Moreover, mAb A3A5 clearly showed dose-dependent inhibitions (Fig.1). Immuno blot analysis showed that mAb A3A5 recognizes a 70KDa peptide of fibronectin (FN), which contains the collagen-binding domain. Polyclonal antisera against FN also supressed the gel contraction especially at an early phase in a concentration-dependent manner (Fig.2). When denatured collagen (gelatin), which has been known to have a higher affinity for FN than collagen, was introduced into the gel, a slight but significant inhibition of the contraction at only early phase was observed (Fig. 3). These results indicate that the role of FN for the fibroblasts to contract collagen gels. However, our previous data indicate that the plasma FN (pFN) is not required for the fibroblast-mediated collagen gel contraction: (1) Removal of pFN from culture medium did not affect the gel contraction; (2) The gel contraction occured also in complete serum-free synthetic media which did not pFN [5]. Therefore, it could be considered that cellular FN (cFN) newly synthesized and secreted by fibroblasts but not pFN plays critical roles for the gel contraction [6].

We found that some kinds of lectins suppress the fibroblast-mediated collagen gel contraction. We introduced the lectins, Concanavalin A (ConA), Lentil Lectin (LCA), Mushroom Lectin (ABA), Pea Lectin (PSA), Peanut Lectin (PNA), Phytohemagglutinin-P (PHA), Pokeweed Mitogen (PWM), Ricinus Communis Lectin-60 (RCA), Soybean Lectin (SBA), and Wheat Germ
Lectin (WGA), into the collagen gel culture of fibroblasts at the concentration 10, 20, or 50 μg/ml. ConA, LCA, PSA, PHA, RCA, and WGA inhibited the gel contraction in the concentration-dependent manner, while ABA, PNA, PWM, and SBA did not (Fig.4). One of the lectins, RCA, which strongly inhibits the gel contraction showed a high toxicity for fibroblasts. The effect of ConA, LCA, PSA, PHA and WGA seemed not to be a result of cytotoxicity, but to have some biological significance, because these lectins were effective in inhibiting the contraction also in the experiment, where fibroblasts were preincubated with the lectins, washed thoroughly with Hanks' solution to remove free lectins and were embedded into collagen gels (Fig.5). These results suggest that these lectins bind the collagen-binding region on the fibroblast-surface glycochain. Interestingly, ConA, LCA, PSA, and WGA inhibited not only fibroblast-mediated gel contraction, but also the spreading of fibroblasts on plastic, while PHA inhibited the gel contraction but did not affect the spreading. PHA may block the specific glycochain for fibroblast-collagen interactions. The characterization of the molecules on the cell surface recognized by PHA or another lectins is now on the way.

In conclusion, there are at least two types of the binding mechanism between fibroblasts and collagen fibrils: (1) Indirect binding via cFN secreted by fibroblasts; (2) Direct binding through glycochain of fibroblast-surface molecules. This conclusion was partly supported by the experiments, shown in Fig. 6, where tunicamysin and monensin, which inhibit, respectively, glycoprotein synthesis, and glycoprotein secretion, suppressed the collagen gel contraction in a reversible and concentration-dependent manner.

Legends to Figures

Fig. 1. Monoclonal antibodies which inhibit the contraction of collagen gels. Fibroblasts (2 x 10⁶) were cultured in 1 ml of collagen gels containing DMEM and 10 % FCS, and IgG of mAbs A3A5 (upper) or B1A4 (lower) at concentrations indicated. (●) Without IgG, (○) 20 μg/ml, (□) 50 μg/ml, (△) 100 μg/ml. Each point represents a single determination.

Fig. 2. Inhibition of the contraction of collagen gels by anti-FN antisera. Fibroblasts (2 x 10⁶) were cultured in 1 ml of 0.1 % collagen gels containing DMEM and the antisera at concentrations indicated. Normal rabbit sera were used as controls. (□ and ■) 10 %, (○ and ●) 20 %, (△ and △) 50 %. Open symbols represent antisera and closed symbols control sera. Each point represents the average of two determinations, the range of which was less than 5 % of the average.

Fig. 3. Significant suppression of the contraction of collagen gels by gelatin. Fibroblasts (2 x 10⁶) were embedded in 0.1 % collagen gels containing DMEM, 10 % FCS and gelatin at concentrations indicated. (●) Without gelatin, (○) 0.1 %, (□) 0.2 %, (△) 0.4 %. Each point represents the average of two determinations, the range of which was within 8 % of average.
Fig. 4. Effect of the lectins on the gel contraction. Fibroblasts (2 x 10^5) were cultured in 0.1% collagen gels containing DMEM, 10% FCS and lectin at concentrations indicated. (●) Without lectins, (▼) 10 µg/ml, (■) 20 µg/ml, (▲) 50 µg/ml.

Fig. 5. Effect of ConA, PHA, WGA, LCA, and PSA, on fibroblast-mediated collagen gel contraction. Fibroblasts (2 x 10^5) were suspended in 1 ml of Hanks' solution containing 1 µg/ml of lectin, pre-incubated at 20°C for 30 min. Fibroblasts were washed with Hanks' solution, and emmbedded into 1 ml of 0.1% collagen gel containing DMEM and 10% FCS. (●) Without lectin-treatment, (●) ConA, (▼) PHA, (■) WGA, (▲) LCA, (●) PSA. Each point represents the average of two determinations, the range of which was less than 6% of the average.

Fig. 6. Effect of tunicamysin and monensin on gel contraction. Fibroblasts (2 x 10^5) were cultured in 1 ml of collagen gel containing DMEM and 10% FCS. Tunicamysin (T) or monensin (M) was introduced into the culture medium at 4 µg/ml (▲), 2 µg/ml (■), 1 µg/ml (▼). (●) Without inhibitors. The arrows indicate the time when each inhibitor was removed. Each point represents the average of two determinations, the range of which was within 5% of the average.

References
Fig. 4.