Effects of Concanavalin A on Chondrocyte Hypertrophy and Matrix Calcification*

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Resting chondrocytes do not usually undergo differentiation to the hypertrophic stage and calcification. However, incubating these cells with concanavalin A resulted in 10–100-fold increases in alkaline phosphatase activity, binding of 1,25(OH)_2-vitamin D_3, type X collagen synthesis, and incorporation of [3H]serine into proteoglycans, and the incorporation of [3H]serine into protein, although they did not affect other lectins tested (including wheat germ agglutinin, lentil lectin, pea lectin, phytohemagglutinin-L, and phytohemagglutinin-E) marginally affected alkaline phosphatase activity, although they activate lymphocytes. Methyllumano side reversed the effect of concanavalin A on alkaline phosphatase activity in articular chondrocyte cultures. ConA and wheat germ agglutinin (WGA) also have been extensively applied in studies of proliferation and differentiation of chondrocyte cultures. In resting chondrocyte cultures, succinyl concanavalin A was as potent as concanavalin A in increasing alkaline phosphatase activity, the incorporation of [35S]sulfate, [3H]glucosamine, and [3H]serine into proteoglycans, and the incorporation of [3H]serine into protein, although concanavalin A did not increase alkaline phosphatase activity in other lectins tested. These results suggest that cell-surface glycoproteins with N-linked sugar chains specific for ConA play crucial roles in chondrocyte differentiation.

In growth plates and bone fracture callus, chondrocytes undergo a sequence of cell changes including proliferation, cessation of cell division, matrix synthesis, maturation (hypertrophy), and calcification. The hypertrophic chondrocytes produce alkaline phosphatase (ALPase), type X collagen (8), and 1,25-dihydroxyvitamin D_3 as well as receptor (9), and they induce matrix calcification (10–13), which is essential for elongation and repair of the skeleton.

Chondrocytes in the resting zone provide a reserve chondrocyte pool in the growth plate, and articular chondrocytes contribute to resilience of the tissue. Neither of these chondrocyte subtypes usually undergoes endochondral ossification. Cells in the resting zone undergo hypertrophy at slower rates than growth-plate chondrocytes when maintained at high density in the presence of 10% serum (9). On the other hand, articular chondrocytes rarely undergo hypertrophy even at high density in the presence of 10% serum (14), although they become hypertrophic in arthritic joints (15). The mechanism by which hypertrophy is suppressed in permanent cartilages is not known. However, lectins may alter the differentiation program in permanent chondrocytes by cross-linking cell-surface glycoproteins, because some surface glycoproteins serve as growth factor receptors, extracellular matrix receptors, and modulators of signal transduction. To test this hypothesis, we examined the effect of 11 lectins with different sugar-binding properties on the expression of maturation-related phenotypes by resting and articular chondrocytes. The results showed that among the tested lectins, ConA alone induces ALPase activity, type X collagen synthesis, vitamin D receptor synthesis, and matrix calcification in resting, but not in articular chondrocyte cultures.

EXPERIMENTAL PROCEDURES

Materials—Lectins, α-methylmannoside, collagenase (Type IA), insulin, retinoic acid, and triiodothyronine (T_3) were purchased from Sigma; Eagle’s medium, α-modification was from Flow Laboratories (McLean, VA); fetal bovine serum was from Life Technologies, Inc.; and fibronectin and type II collagen were from Koken Co. (Osaka, Japan). Human bone morphogenetic protein-2 (BMP-2, recombinant) was a gift of Dr. J. M. Wozney (Genetics Institute, Cambridge, MA) and Dr. K. Takahashi (Yamanouchi Pharmaceutical Co., Tokyo). Human transforming growth factor-β (TGF-β, recombinant) and human insulin-like growth factor-I (IGF-I, recombinant) were purchased from Wako Pure Chemical (Osaka, Japan). Human calcitonin was purchased from Peninsula Laboratories Inc. (Belmont, CA). 1,25(OH)_2-vitamin D, 1,25-dihydroxy[26,27-methyl-3H]cholecalciferol (180 Ci/mmol), and parathy-

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The abbreviations used are: ConA, concanavalin A; PHA, phytohemagglutinin; WGA, wheat germ agglutinin; T_3, triiodothyronine; PTH, parathyroid hormone; PAGE, polyacrylamide gel electrophoresis; Me-Man, methylmannoside; TGF-β1, transforming growth factor β-1; IGF-I, insulin-like growth factor-I; ALPase, alkaline phosphatase.

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Chondrocyte Cultures—Chondrocytes were isolated from the growth plate and resting cartilage of ribs of 4-week-old male Japanese White rabbits, as described (14, 16). Unlike growth plates of long bones, the rib growth plate does not contain resting chondrocytes. The resting cell population is not contaminated by growth-plate chondrocytes, although the growth-plate cell population may be partially contaminated by few (<10%) resting chondrocytes (9). The majority of “growth-plate chondrocytes” start to proliferate after seeding and recapitulate the differentiation program after cell division stops, even though they originate from different (proliferating, matrix-forming, and hypertrophic) zones (10).

Articular chondrocytes were isolated from the surface (0.2 mm) of the articular cartilage from the femur at knee joints of the same rabbits, since the deep zone contains maturing chondrocytes (14).

Cells were seeded at 10^3 or 3 × 10^5 cells/6-mm plastic microwell, and grown in 0.1 ml of Eagle’s medium, α-modification, supplemented with 10% fetal bovine serum, 50 μg/ml ascorbic acid, 32 units/ml penicillin, and 40 μg/ml streptomycin (Medium A). Alternatively, cells were seeded at densities of 2 × 10^5 cells/35-mm dish and 10^5 cells/100-mm dish and 10 ml of Medium A, respectively. The cultures were supplied with fresh Medium A every other day. After reaching confluence in 6-, 35-, and 100-mm dishes (day 5 or 7), the cells were transferred to Eagle’s medium, α-modification in the presence of various lectins and 0–2% fetal bovine serum for 6 or 24 h, after which the cell layers were washed three times with Medium A. Because lectins bind to serum glycoproteins, the serum concentration was reduced during the incubation with lectins for 6–24 h. Thereafter, the cells were maintained for 0–28 days in Medium A, unless otherwise specified.

Determination of ALPase Activity—Chondrocytes were seeded at 10^4 or 3 × 10^5 cells/6-mm well, and maintained in Medium A. On days 5 or 7, they were transferred to α-modified Eagle’s medium supplemented with 2% fetal bovine serum in the presence or absence of lectins or growth factors for 24 h. Thereafter, the cells were maintained for 0–28 days in Medium A in the presence of lectins and in the presence or absence of growth factors. The media were replaced every other day. Unless otherwise specified, lectins were added to the cultures only once on day 5 or 7, whereas growth factors were added to the cultures every other day from day 5 or 7.

Cells were disrupted in a glass homogenizer in 1 ml of 0.9% NaCl, 0.2% Triton X-100 at 0–4 °C and centrifuged at 15 min, 12,000 g at 0–4 °C. The precipitation of ALPase activity in the supernatant, which accounted for 95% of the total, was assayed in 0.5 M Tris-HCl buffer (pH 9.5) supplemented with 0.5 mM p-nitrophenyl phosphate and 0.5 mM MgCl₂ (17).

Determination of DNA, Protein, and Hexuronic Acid—The DNA content was determined by means of a fluorometric procedure (18). Total protein was determined by dye binding (19). Hexuronic acid was determined by means of a fluorometric procedure (18). Total protein and DNA synthesis were determined by measuring the incorporation of [3H]serine and [3H]thymidine into 5% trichloroacetic acid-insoluble cell precipitates, respectively (24).

Results

Effect of Lectins, Hormones, and Growth Factors on ALPase Activity in Chondrocytes—Resting chondrocytes in high or low density cultures were exposed for 24 h to ConA on day 5 or 7, respectively, after the cultures reached confluence. Thereafter, they were maintained in the presence of 10% serum for 10 days. ConA (10 μg/ml) was added to the cultures only once on day 5 or 7, because ConA bound to the cells and extracellular matrix macromolecules within 24 h. The incubation with ConA caused a >12-fold increase in ALPase activity in high and low density cultures on days 15 and 17, respectively (Fig. 1).

Among growth factors and hormones, BMP, thyroid hormone, calcitonin, and retinoic acid increase ALPase and/or type X collagen synthesis by chondrocytes (25–30). These compounds were determined by incubating the cells with a 200-fold excess of unlabeled 125(I)-OH- vitamin D.

Determination of 45Ca Uptake and Calcium and Alizarin Red Staining—Chondrocytes in 35-mm dishes were exposed to 45CaCl₂ (20 μCi/ml) in 0.2 ml of α-modified Eagle’s medium 3 h before the end of the incubation. Cell-matrix layers were homogenized in 0.9% NaCl, 0.2% Triton X at 0 °C, then centrifuged for 15 min at 12,000 × g. The precipitate was washed with 0.1 M CaCl₂ in 0.005 M Tris-HCl, pH 7.4, at 20 °C for 30 min to remove exchangeable 45Ca, then solubilized by incubation in 0.5 M HCl for 3 h at 20 °C (21). The levels of 45Ca were determined by atomic absorption spectrophotometry (model AA-640; Shimadzu, Kyoto, Japan). The cell-matrix layers were stained with alizarin red-S as described (22).

Analysis of Secreted Proteins and Amino Acid Sequence—Resting chondrocytes were seeded at a density of 10^6 cells/100-mm dish, and maintained in Medium A. On days 7, 12, and 17, the cells were exposed or not to 10 μg/ml ConA in the absence of serum for 6 h. They were incubated in 10 ml of Medium A with 10% serum from days 7 to 12 and from days 15 to 17, and in 10 ml of α-modified Eagle’s medium without serum from days 12 to 15 and 17 to 20. Proteins in the conditioned media on days 12–15 and 17–20 were separated by SDS-PAGE, then stained with silver nitrate. Alternatively, proteins in the media were transferred electrophoretically to a polyvinylidene difluoride membrane (23), and stained with Coomassie Brilliant Blue. The N-terminal amino acids of a 15-kDa protein were determined using an automatic protein sequence (Applied Biosystems model 476A).

Determination of Proteoglycan, Protein, and DNA Synthesis—When resting chondrocyte cultures in 6-mm wells became confluent, they were incubated in 0.1 ml of Dulbecco’s modified Eagle’s medium containing 0.3% fetal bovine serum, 32 units/ml penicillin, and 40 μg/ml streptomycin (Medium B). They were then incubated in 0.1 ml of fresh Medium B with or without ConA for 6–24 h. The media were replaced every other day. Unless otherwise specified, lectins were added to the cultures only once on day 5 or 7, whereas growth factors were added to the cultures every other day from day 5 or 7.

Cells were disrupted in a glass homogenizer in 1 ml of 0.9% NaCl, 0.2% Triton X-100 at 0–4 °C and centrifuged at 15 min, 12,000 g at 0–4 °C. The precipitation of ALPase activity in the supernatant, which accounted for 95% of the total, was assayed in 0.5 M Tris-HCl buffer (pH 9.5) supplemented with 0.5 mM p-nitrophenyl phosphate and 0.5 mM MgCl₂ (17).

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Coating the Culture Dishes—Plastic tissue culture dishes (diameter, 35 mm) were incubated with 1 ml of 0.1 M NaHCO₃ containing type II collagen (10 μg/ml) or fibronectin (10 μg/ml) at 37 °C for 3 h, then washed three times with saline.

Cell Adhesion—Chondrocytes in primary cultures were harvested with phosphate-buffered saline containing 0.01% trypsin and 0.01% EDTA, then seeded at a density of 2 × 10⁶ cells/35-mm plastic, fibronectin- or collagen-coated well. They were incubated with lectins at 37 °C in 2 ml of Dulbecco’s modified Eagle’s medium for 3 h before being photographed.
greater than that of BMP-2, IGF-I, or T3 (Fig. 1; data not shown). The effect of retinoic acid and calcitonin on ALPase was much lower than that of ConA.2

The effect of ConA on ALPase in resting chondrocyte cultures was detectable at 1 μg/ml and maximal at 5–10 μg/ml (Fig. 3A). ConA at 10 μg/ml increased ALPase activity in resting chondrocytes 50-fold (Fig. 3A), whereas it increased ALPase activity in growth-plate chondrocytes only 1.3-fold because of a high basal level (Fig. 3B).

The lectin specificity of the ConA effect on ALPase induction was examined by exposing resting chondrocytes to the various lectins that activate lymphocytes. A divalent derivative of ConA (succinyl ConA) was as potent as the native tetravalent ConA in stimulating ALPase activity (Fig. 3, A and B). Lentil lectin, garden pea lectin (pea), and Phascolus vulgaris agglutinin-E (PHA-E) induced very low levels of ALPase activity (Fig. 3A). Maclura promifera, lima bean lectin, WGA, Scotch broom lectin, Ulex europaeus agglutinin (1 + II), lotus lectin, and P. vulgaris agglutinin-L (PHA-L) did not affect ALPase activity (Fig. 3A).

Effects of ConA on the Levels of DNA, Uronic Acid, and ALPase in Chondrocyte Cultures—Fig. 4 shows the time courses of the increases in DNA, uronic acid, and ALPase contents in cultures exposed or not to ConA. Resting chondrocytes seeded at 10^4 cells/6-mm well well proliferated for two generations, yielding a confluent monolayer on day 5, then underwent one or two rounds of cell division to form a multi-cell layer by day 10. The DNA content reached a plateau on day 16 (Fig. 4A). Exposing chondrocytes to ConA for 24 h on day 5 suppressed DNA synthesis on days 6, 8, and 10 (Fig. 4A). The cells exposed to ConA started to proliferate from day 14, then the DNA content reached the level in cultures not exposed to ConA by day 24 (Fig. 4A).

Most of the macromolecules containing uronic acid synthesized by chondrocytes exposed or not to ConA are large, chondroitin-sulfate proteoglycans (aggrecan) (5). ConA selectively stimulates aggrecan synthesis without increasing the synthesis of hyaluronic acid and small proteoglycans (5). In ConA-free cultures, the uronic acid level increased from day 10, and reached a plateau on day 18 (Fig. 4B). In cultures exposed to ConA, the level promptly increased from day 5–6, and reached a maximum on day 8. Thereafter, it decreased gradually, and returned to the level of ConA-free cultures on day 20. On day 8, the uronic acid level in the exposed cultures was 4-fold higher.
than that in ConA-free cultures.

In ConA-free chondrocyte cultures, the level of ALPase activity was very low throughout a 24-day culture period (Fig. 4C). However, upon exposure to ConA, it markedly increased from day 8, and reached a maximum on day 14 (Fig. 4C). The high ALPase level was sustained at least until day 24. Some cultures were exposed twice to ConA on days 5 and 14. The second exposure on day 14 increased ALPase activity 1.4-fold within 48 h,2 even though the uronic acid content reached a plateau on day 8. These findings suggested that the ConA induction of ALPase is not secondary to the accumulation of proteoglycan.

Incubation of chondrocytes with ConA for 6 h had the same effects on uronic acid and ALPase levels as that for 24 h, 2 perhaps because the lectin binds to the cell-matrix layer within 6 h.

Effects of Methyl-α-mannopyranoside (MeMan) on ALPase Activity—The addition of MeMan on day 5 for 24 h suppressed the effect of ConA on ALPase activity dose-dependently with an ED₅₀ of 10 μM (Fig. 5A). MeMan alone had little effect on ALPase activity. Furthermore, the addition of MeMan on day 12 reversed the effect of ConA on ALPase activity within 48 h, suggesting that prolonged action of the lectin is required for ALPase synthesis after the accumulation of proteoglycan. In contrast, the addition of MeMan on day 16 had little effect on ALPase activity (Fig. 5B), perhaps because the ConA-activated cells had already differentiated to the irreversible hypertrophic stage.

Effect of ConA on the Chondrocyte Size—Because ConA was removed gradually from the cell-matrix layer (Fig. 4), chondrocytes were incubated three times with ConA on days 7, 12, and 17. Resting chondrocytes exposed to ConA (Fig. 6B), but not the untreated cells (Fig. 6A), became hypertrophic by day 20. On the other hand, articular chondrocytes became fibroblastic in the absence of ConA by day 20 (Fig. 6C), because of the low seeding cell density and the low serum concentration used in this study. Nevertheless, the articular chondrocytes exposed to ConA maintained a spherical phenotype, although they did not become hypertrophic (Fig. 6D). When articular chondrocytes were seeded at high density (10⁴ cells/6-mm plastic microwell) and maintained in the presence of 10 μg/ml ConA, the resting chondrocytes exposed to ConA (B, 1279 ± 591 μm²) were 2.5- and 3-fold larger than those that were not (A, 506 ± 176 μm²) and articular chondrocytes (D, 385 ± 85 μm²), respectively. The diameter of resting chondrocytes exposed to ConA (B, 39.0 ± 10.2 μm) was similar to that (30–40 μm) of hypertrophic chondrocytes in the growth plate in vivo.2

Induction of Type X Collagen in ConA-exposed Chondrocytes—Resting chondrocytes maintained without ConA synthe-
sized type II collagen but not type X collagen, a marker of hypertrophic chondrocytes. However, ConA-exposed chondrocytes synthesized both type II and X collagens on day 15 (Fig. 7).

**Induction of 1,25(OH)2-Vitamin D Receptor in ConA-exposed Chondrocytes—**

Next we examined whether ConA stimulated the expression of the other maturation-related phenotypes in resting chondrocyte cultures. Resting chondrocytes maintained without ConA showed only a low level of the 1,25(OH)2-vitamin D binding capacity on days 14 and 20 (Fig. 8). However, exposure to ConA on day 7 resulted in 30- and 5-fold increases in the binding of 1,25(OH)2-vitamin D to chondrocytes on days 14 and 20, respectively.

**Calcification—**

No calcification was evident in ConA-free cultures of resting chondrocytes. However, exposure to ConA for 24 h on day 7 resulted in 10–20-fold increases in 45Ca incorporation into insoluble material and the calcium content on day 35 (Fig. 9, A and B). The cell-matrix layer of chondrocytes exposed to ConA was intensely stained with alizarin red (Fig. 9 C).

**Analysis of Secreted Proteins—**

ConA had little effect on the SDS-PAGE profiles of secreted proteins in the media conditioned by resting chondrocytes, except that it markedly increased the level of 15-kDa protein (Fig. 10). N-terminal analysis of the 15-kDa protein revealed a 28-amino acid sequence of LLGGLEDVDAQEKDVQRALGFAESSYNK, which was homologous to that of rat, bovine, mouse, and human cystatin C (61, 57, 57, and 50% identity, respectively) and human cystatins D, SA, S, and SN (54, 54, 58, and 54% identity, respectively). The molecular mass of the 15-kDa protein was also similar to that of cystatin. It is unlikely however, that the cystatin-like protein mediates the action of ConA on chondrocytes, because this protein partially purified from the conditioned medium had little effect on ALPase activity.3 The media

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3 M. Nishimura, unpublished data.
conditioned by ConA-treated chondrocytes did not increase ALPase activity in resting chondrocyte cultures, suggesting that the ConA stimulation of ALPase is not mediated by secreted factors.

Comparison between the Effects of ConA and Succinyl ConA on Protein Synthesis, Proteoglycan Synthesis, DNA Synthesis, and Cell Spreading—Native, tetravalent ConA stimulates lymphocyte proliferation at lower concentrations than the divalent ConA derivative, succinyl ConA (33). However, in chondrocyte cultures, succinyl ConA was as potent as ConA in stimulating the incorporation of [3H]glucosamine (Fig. 12A), and [3H]serine (Fig. 11C) into proteoglycans and the incorporation of [3H]serine into total protein (Fig. 11D). On the other hand, succinyl ConA suppressed DNA synthesis to a lesser extent than ConA (Fig. 11E). The inhibition of DNA synthesis by these lectins was dose-dependently reversed by MeMan (Fig. 11E). The effect of ConA on proteoglycan synthesis was also reversed by MeMan (5).

Succinyl ConA and ConA differed with respect to their actions on chondrocyte morphology. Resting chondrocytes assumed a fibroblastic configuration in monolayer cultures at a low serum concentration (0.3%). ConA at 10 μg/ml remarkably changed the cells from fibroblastic to spherical within 24 h (5), whereas succinyl ConA induced a moderate change from fibroblastic to polygonal at 48–96 h, perhaps because of the increase in the proteoglycan content. ConA, but not succinyl ConA, may directly alter the architecture of the cytoskeleton.

To test this notion, chondrocytes were seeded on fibronectin-coated dishes and incubated for 3 h in the absence or presence of ConA. ConA-free cells fully spread on the substrate within 3 h (Fig. 12A). ConA inhibited the formation of long and/or thin cellular projections at 1–3 μg/ml (Fig. 12, B and C), and abolished cell spreading at 10 μg/ml (Fig. 12D). In contrast, succinyl ConA had little effect on cell spreading at 30 μg/ml (Fig. 12E). Similar results were obtained with chondrocytes seeded on plastic tissue culture dishes and type II collagen-coated dishes.

**DISCUSSION**

Exposure to ConA markedly increased ALPase activity and the size of resting chondrocytes. ALPase synthesis was stimulated more by ConA than by hormones and growth factors that supposedly enhance ALPase synthesis by chondrocytes. The specificity of the ConA action on ALPase was indicated by the following observations. (i) ConA did not stimulate ALPase synthesis and hypertrophy in articular chondrocyte cultures. The marginal effect of ConA on ALPase activity in growth-plate chondrocyte cultures could have been due to contamination of the growth-plate cell population by resting chondrocytes. (ii) Lentil and garden pea lectins had little effect on ConA activity, although their monosaccharide-binding properties (mannon/glucose) are similar to those of ConA. The crystal structure of pea lectin is also similar to that of ConA (34). However, the oligosaccharide-binding properties of lentil and garden pea lectins differ from those of ConA (1). (iii) Of the lectins examined, only ConA stimulated chondrocyte hypertrophy and calcification. This is important, because many lectins with different sugar-binding properties activate lymphocytes. (iv) ALPase induction by ConA was suppressed by methylmannoside.

Our finding indicated that ConA does not randomly activate gene expression by chondrocytes, but that it initiates the maturation program in resting chondrocytes. Exposing resting chondrocytes to ConA for 6–24 h suppressed cell division, then increased the proteoglycan content, the cell size, ALPase activity, the vitamin D receptor level, the incorporation of 45Ca into insoluble material, and the calcium content. These cell-matrix changes proceeded in the same order as that seen in growth plates and bone fracture callus in vivo. The cell size as well as the ALPase and the vitamin D receptor levels in the ConA-exposed chondrocyte cultures were comparable to those in the
We postulated that specific lectins induce the maturation of these cells. They were exposed to \([35S]\)sulfate (measuring the incorporation of \([35S]\)sulfate before the end of incubation. Proteoglycan synthesis was estimated by

\[ tate, \text{respectively. The values are averages } \pm \text{S.D. of four cultures.} \]

**Changes in cell-matrix interactions and/or growth factor levels in vivo growth plate can also become hypertrophic when cultured at high density in the absence of a tissue culture surface (9).**

Resting cartilage provides the reserved exhaustion pool for the growth plate while remaining flexible, whereas articular cartilage supports high compressive loads in connective tissues. Thus, extensive cross-linking of cell-surface glycoproteins is required for the rapid cell-shape change, but not for chondrocyte differentiation.

Although ConA and WGA activate insulin receptors in adipocytes (2–4), insulin induced only a very low level of ALPase activity in chondrocytes. Thus, the stimulation of ALPase is not mediated by the binding of ConA to insulin receptors. In addition, ConA did not down-regulate PTH, TGF-β1, and fibroblast growth factor receptors in chondrocytes.

ConA enhanced the synthesis of 15-kDa cystatin-like protein by resting chondrocytes. Since ConA also enhanced this synthesis by articular chondrocytes, the cystatin-like protease inhibitor is unlikely to mediate the action of ConA on chondrocyte hypertrophy. Previous studies have shown that ConA stimulates and inhibits the synthesis of matrix-metalloproteinases and TIMP inhibitor, respectively, by fibroblasts (35). These findings suggest that some ConA-binding surface glycoproteins are involved in the control of matrix breakdown in connective tissues.

**Interactions of chondrocytes with artificial substrate impaire their phenotypic expression. Thus ConA may enhance proteoglycan and ALPase synthesis via inhibition of their spreading on tissue culture surface.** We tested this notion by comparing the action of ConA with that of succinyl ConA. Tetravalent ConA induced extensive cross-linking of membrane glycoproteins, whereas the divalent ConA derivative did not. This accounted for the differences between the actions of ConA and succinyl ConA on lymphocytes (36). In this study, ConA, but not succinyl ConA, inhibited the spreading of chondrocytes, although these lectins exerted the same effects on ALPase activity, proteoglycan synthesis, and total protein synthesis. Thus, extensive cross-linking of cell-surface glycoproteins is required for the rapid cell-shape change, but not for chondrocyte differentiation.

The tertiary structures of ConA and the ConA-sugar complex, as well as its sugar-binding site, have been characterized (37). In addition, the oligosaccharide structure required for ConA binding has been determined (38), and a family of peptides that mimic the binding of MeMan to ConA have been identified from screening a hexapeptide epitope library (39). This information will be useful for future studies on the action of ConA on animal cells.

In conclusion, the present study showed that ConA selectively acts on resting chondrocytes to initiate the maturation program, and that the ConA stimulation of chondrocyte differentiation is not directly linked with lectin-induced changes in the cytoskeleton. Resting chondrocytes exposed to ConA may

**FIG. 11. Effects of ConA and succinyl ConA on proteoglycan synthesis, total protein synthesis, and DNA synthesis.** When resting chondrocyte cultures in 6-mm wells reached confluence, the cells were incubated in 0.1 ml of medium containing 0.3% fetal bovine serum (Medium B). They were then incubated in 0.1 ml of fresh Medium B containing various concentrations of lectins for 24 h (A–D). Alternatively, they were incubated in 0.1 ml of fresh Medium B containing various concentrations of MeMan, 10 μg/ml lectin, or both for 24 h (E). These cells were exposed to \([35S]\)sulfate (A) for 17 h, \([3H]\)glucosamine (B) for 6 h, \([3H]\)serine (C and D) for 6 h, or \([3H]\)thymidine (E) for 3 h before the end of incubation. Proteoglycan synthesis was estimated by measuring the incorporation of \([35S]\)sulfate, \([3H]\)glucosamine, or \([3H]\)serine into material precipitated with cetylpyridinium chloride after digestion with Pronase E. Total protein synthesis (D) and DNA synthesis (E) were determined by measuring the incorporation of \([3H]\)serine and \([3H]\)thymidine into 5% trichloroacetic acid-insoluble cell precipitate, respectively. The values are averages \pm S.D. of four cultures.

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**FIG. 12. Effects of ConA and succinyl ConA on chondrocyte spreading.** Resting chondrocytes were seeded onto fibronectin-coated dishes and incubated in the presence of 0 (A), 1 (B), 3 (C), and 10 μg/ml (D) ConA or 30 μg/ml succinyl ConA (E) for 3 h.
be useful as a novel model for studies on endochondral bone
formation.

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