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Antigenic and immunogenic properties of chondrocytes. Implications for chondrocyte therapeutic transplantation and pathogenesis of inflammatory and degenerative joint diseases

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Abstract

In physiological conditions chondrocytes are protected from contact with immunocompetent cells by the extracellular matrix, and transplanted fragments of allogeneic cartilage are not rejected. Cartilage produced by allogeneic chondrocytes, however, evokes the immune response of the recipient and is gradually destroyed. Immunisation by allogeneic chondrocytes is induced by the contact of their surface molecules with cells of the immune system. Chondrocytes constitutively express class I and, in some species, class II major histocompatibility complex (MHC) molecules. Expression of MHC class II molecules is induced in vitro by pro-inflammatory cytokines and in vivo in the course of the rejection of transplanted allogeneic cartilage. Low level of MHC class II molecules is found on the surface of human articular chondrocytes in patients with rheumatoid arthritis and osteoarthritis. Cartilage produced by transplanted allogeneic chondrocytes is destroyed by monocytes/macrophages and cytotoxic T and natural killer (NK) cells. NK cells show spontaneous cytotoxic reactivity against isolated chondrocytes and participate in the rejection of transplanted isolated chondrocytes. Chondrocytes express molecules that can serve as potential antigens in inflammatory joint diseases. Chondrocytes express cartilage-specific membrane antigen (CH65), human cartilage glycoprotein-39 (HC gp-39), hyaluronan binding adhesion molecule CD44, thymocyte antigen-1 (Thy-1) – CD90, signal transducer – CD24, lymphocyte function-associated antigen-3 (LFA-3) – CD58, and type I transmembrane protein Tmp21. On the other hand, although chondrocytes express major histocompatibility complex (MHC) class I and class II molecules, they can also exert immunosuppressive and immunomodulatory effects on immunocompetent cells. Isolated chondrocytes do not trigger an efficient allogeneic immune response in vitro and suppress, in a contact-dependent manner, proliferation of activated T cells. This suppression is associated with the expression by chondrocytes of multiple negative regulators of immune response. Chondrocytes express programmed death-ligand (PD-L), chondromodulin-I and indoleamine 2,3-dioxygenase (IDO), molecules that promote self-tolerance and suppress the immune system.

Key words: *chondrocyte antigens, chondrocyte immunogenicity, chondrocyte transplants, chondrocyte antigens in joint diseases, chondrocyte-mediated immunomodulation, chondrocyte-mediated immunosuppression.*

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Introduction

Clinical application of chondrocyte transplants and possible involvement of chondrocyte antigens in rheumatoid arthritis (RA) and other inflammatory joint diseases stimulated enormously studies on the expression of class I and II major histocompatibility complex (MHC) molecules by chondrocytes as well as expression of other chondrocyte surface molecules that might stimulate antibody production. Thus, it appeared that the short recapitulation of the present status of chondrocyte as a player in joint surface reconstruction,

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and possibly the villain in inflammatory joint disease, would be indicated. Because the first chondrocyte transplants were done in our laboratory [1] and our review covering various aspects of chondrocyte antigenicity published in 2002 [2] is now obsolete, we felt that we should write a new survey of these topics as a continuation of laboratory tradition.

Cartilage as immunoprivileged tissue

In physiological conditions chondrocytes are sequestrated from the cells of the immune system by extracellular matrix, and transplanted fragments of allogeneic cartilage are not rejected [3]. However, in inflammation processes, after trauma or after transplantation of isolated allogeneic chondrocytes, their surface molecules are exposed to the contact with immunocompetent cells, and chondrocytes initiate the immune response [2, 4]

Major histocompatibility complex (MHC) molecules expression on chondrocytes

Isolated chondrocytes transplanted into autogenic, syngeneic, or allogeneic hosts formed cartilage similar to that from which they originate [1, 5]. Syngeneic chondrocyte grafts did not show any signs of rejection [6, 7], but cartilage produced by allogeneic chondrocytes evoked the immune response of the recipient and was gradually destroyed [8, 9]. Immunisation by allogeneic chondrocytes could be induced by MHC molecules present on the surface of chondrocytes [10, 11]. The presence of MHC class I molecules (also called human leukocyte antigens HLA-A, HLA-B, HLA-C) has been found on the surface of human chondrocytes derived from healthy individuals [12-14]. The expression of MHC class II molecules was observed on the surface of normal rabbit articular chondrocytes [11]. Malejczyk and Romaniuk [15] found that rat articular chondrocytes expressed MHC class II molecules encoded by the RT1D subregion without the presence of molecules encoded by the RT1B subregion. Human unstimulated articular and nasal chondrocytes have been shown to lack MHC class II molecules [12, 14, 16-18]. Expression of these molecules could be induced *in vitro* under the influence of pro-inflammatory cytokines, for example interferon-γ (IFN-γ) [12, 14, 16, 19], and *in vivo*, in the course of the rejection of transplanted allogeneic cartilage [17]. Moreover, the presence of MHC class II molecules (HLA-DR, HLA-DP, HLA-DQ) was found on the surface of human articular chondrocytes from patients with rheumatoid arthritis (RA) and osteoarthritis (OA), but they did not occur on the surface of chondrocytes in patients with osteochondroma [12, 20]. Abe *et al.* [21] demonstrated, that chondrocytes from osteoarthritic knees expressed MHC class I molecules, but only 1 to 2% of chondrocytes expressed MHC class II molecules.

The presence of class II MHC molecules, usually found on professional antigen presenting cells, could allow chondrocytes to present antigens to T cells. It has been shown that rabbit isolated articular chondrocytes preincubated with ovalbumin have been able to present ovalbumin to lymph node cells obtained from rabbits immunised with this protein [11]. A similar effect was observed also for human articular and nasal chondrocytes which, after stimulation with IFN-γ, presented tetanus toxoid and were able to stimulate proliferative response of T lymphocytes [17, 22].

Monoclonal antibody evaluation of cells infiltrating the allocartilage in rats showed that the main cells actively participating in cartilage destruction were monocytes/macrophages and cytotoxic T and natural killer (NK) cells [9]. Moreover, RT1.B class II MHC molecules appeared on some chondrocytes after transplantation to an allogeneic recipient, and their expression increased in the course of rejection and could be associated with the activity of pro-inflammatory cytokines produced by infiltrating cells [15]. This expression, necessary for the presentation of antigens, might be important for initiation of the specific immune response and might explain the strong reaction to isolated allogeneic chondrocyte transplants [16, 17, 20].

Natural cytotoxicity against chondrocytes

Natural killer cells may play an important role in the rejection of transplanted isolated chondrocytes and in cartilage destruction observed in the course of inflammatory joint diseases. Spontaneous cytotoxic reactivity was observed against isolated mice epiphyseal and rat epiphyseal, costal, nasal, and auricular chondrocytes [23, 24]. Similar, but weaker, cytotoxicity against human chondrocytes isolated from mature articular cartilage has also been observed by Yamaga [25]. The activity of NK cells in the rejection of transplanted isolated chondrocytes was confirmed by Sommaggio *et al.* [26]. They discovered that cytotoxic activity of NK cells against chondrocytes was associated with the expression on chondrocyte ligands for human NK cells – natural cytotoxicity triggering receptor 3 (NKp30, CD337) and natural cytotoxicity triggering receptor 1 (NKp46, CD335), and that the adhesion of NK cells and chondrocytes was regulated by pro-inflammatory cytokines and was dependent on the expression of vascular cell adhesion molecule 1 (VCAM-1, CD106) and intercellular adhesion molecule 1 (ICAM-1, CD54) on chondrocytes. Additionally, constitutive expression of ligand for NK cell natural cytotoxicity triggering receptor 2 ligand (NKp44L) by normal human articular chondrocytes was confirmed by Białoszewska *et al.* [27]. NKp30, NKp44 (CD336), and NKp46 are natural cytotoxicity receptors (NCRs) of NK cells, which can bind some ligands on the surface of target cells, for example highly charged HS/heparin structures [28].

NK cells are also involved in the cartilage destruction process in the course of inflammatory autoimmune diseas-

es [25, 29, 30]. Yamaga *et al.* [25] studied the capability of lymphocytes from healthy individuals and patients with arthritis to lyse chondrocytes. They found that peripheral blood mononuclear cells (PBMC) from healthy individuals possessed only a low ability to lyse chondrocytes, whereas cells from the synovial fluid of patients with RA showed lytic activity toward chondrocytes. This chondrolytic activity of lymphocytes was greatly increased by interleukin-2 (IL-2). In contrast, treatment of chondrocytes with IFN-γ, which enhanced MHC class I and II gene expression, decreased the susceptibility of chondrocytes to lysis. The activity of NK in the pathogenesis of inflammatory joint diseases was confirmed by Dalbeth and Callan [29], who observed NK cells present within inflamed joints.

The mechanisms responsible for recognition of chondrocytes by NK cells during cartilage destruction observed in inflammatory joint diseases are still poorly understood. Białoszewska *et al.* [31] found that chondrocyte sensitivity to lysis by NK cells was dependent on the chondrocyte-specific phenotype of target cells. The authors showed that the lysis of rat epiphyseal chondrocytes was regulated by the surface expression of chondroitin sulphate, one of proteoglycans reported as a ligand for NK cell receptors [32]. The same group of researchers [33] found that IL-2 stimulated human articular chondrocyte expressed lectin-like transcript-1 (LLT1) molecules. Interactions between killer cell lectin-like receptor subfamily B member 1 (NKR-P1A, CD161) on NK cells and LLT1 on target cells inhibited NK cell-mediated cytotoxicity [33, 34].

Immunogenic properties of chondrocytes – implications for chondrocyte therapeutic transplantation

Autologous chondrocyte transplantation

The capacity of articular cartilage to repair is limited, and cartilage injuries that reach subchondral bone are usually filled by fibrous tissue formed by cells migrating from bone marrow [35, 36]. For the first time, autogenic articular chondrocytes isolated from the non-weight-bearing areas of cartilage and expanded in vitro have been used for treatment of patients with deep articular cartilage defects [37]. This method has been constantly modified in recent years, and cultured autogenic chondrocytes have been successfully applied for healing articular cartilage defects in humans [38, 39]. Some new methods are associated with the use of cell-seeded scaffolds, for example matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI), a new operation procedure using a cell-seeded collagen matrix applied for the treatment of localised full-thickness cartilage defects [40, 41]. The use of chondrocytes for the treatment of patients with deep articular cartilage defect is hampered. Chondrocytes liberated from cartilage matrix and placed in monolayer culture undergo a transition from chondrocyte phenotype to fibroblastoid phenotype. They change the profile of the macromolecules produced by suppressing collagen type II and proteoglycan synthesis and by the concomitant expression of collagen type I [42-45]. Transplantation of these dedifferentiated cells into cartilage defect causes the growth of fibrous cartilage [46]. These changes can be reverted by transferring chondrocytes from monolayer to 3-D culture in agarose gel [47], alginate beads [48], or on the surface coating with collagen type I or IV [49].

Allogeneic chondrocyte transplantation

It is necessary to emphasise the basic disadvantages of autogenic chondrocyte transplantation – the need for surgical intervention and the limited availability of autogenic cells, especially in older donors. Allogeneic chondrocytes are more accessible, but cartilage produced by them in articular surface defects in rats was resorbed by infiltrating immune cells [50, 51]. This acute rejection of newly formed allocartilage was not prevented even by the strong immunosuppressive agents cyclosporine A (CsA) and cladribine (2-chlorodeoxyadenosine) [52]. Allogeneic cartilage formed by chondrocytes transplanted into articular cartilage defects was infiltrated in its deep part, submerged in subchondral bone and bone marrow, while on the surface of the transplant facing the joint cavity infiltrating cells were absent [50, 52]. This suggested that immunisation of recipients occurred via the contact of chondrocytes with bone marrow cells. Moskalewski *et al.* [53] showed, that separation of transplanted allogeneic chondrocytes from the contact with bone marrow cavity prevented immunisation of recipients and the cartilage remained free of infiltration.

In subsequent studies, it was shown that cartilage formed by transplanted syngeneic chondrocytes in joint surface defects or intramuscularly was rejected in animals sensitised with allogeneic chondrocytes. These transplants were infiltrated by $CD8^+$ lymphocytes, which accumulated close to the transplants and invaded only their peripheral part, and the macrophages penetrating into the transplants [7, 54]. The rejection of cartilage formed by syngeneic chondrocytes transplanted into sensitised recipients might indicate the presence surface tissue-specific chondrocyte antigens.

Tissue-specific chondrocyte antigens

The expression of tissue-specific chondrocyte antigen(s) has been suggested since Langer *et al.* [55] found that rats injected with syngeneic chondrocytes developed autoreactivity, assayed by leukocyte migration test. Lately it has been shown that both rat allogeneic and syngeneic chondrocytes stimulated proliferation of lymphocytes in mixed lymphocyte-chondrocyte cultures [56, 57], and these observations were confirmed with human, bovine,

and canine cells [58]. Furthermore, Malseed and Heyner [59] and Lance *at al*. [57] reported that sera from rabbits immunised with rat chondrocytes contained specific antibodies against chondrocytes, and these immunoglobulins showed cross-reactivity with syngeneic chondrocytes [57].

In subsequent years, it has been found that in patients with RA and OA, different types of antibodies react specifically with the chondrocyte surface [17, 60-62]. Some of these antibodies are directed against type II collagen [63], as well as types IX and XI [17], suggesting the presence of a collagen molecule on the surface of chondrocytes. Among the theories explaining the presence of collagen on the surface of chondrocytes, the most likely assumption is that collagen molecules are bound to the cell membrane by the appropriate receptors [63]. Several potential collagen receptors have been detected on the surface of the chondrocytes. One of them is anchorin C II, a 31 kDa glycoprotein, belonging to the annexin family, which may bind type I, II, III, V and X collagen [64, 65]. Another receptor for collagen is colligin, a 47 kDa glycoprotein that can bind to collagen type I and IV [66]. Other molecules that are candidates for collagen membrane receptors are syndecan [67], chondronectin [68] and integrins [69].

Another group of antibodies present in RA and OA patients are antibodies that do not react with type II collagen but are directed against other chondrocyte surface proteins, which can be considered as tissue-specific chondrocyte antigens. It has been shown that about 32% of the sera of the RA patients contained antibodies, which reacted with antigens expressed exclusively by chondrocytes, and 75% of these sera contained antibodies against antigens expressed by both chondrocytes and fibroblasts [70]. These surface molecules could act as targets in inflammatory joint diseases.

Chondrocyte antigens as potential targets in inflammatory joint diseases

Cartilage-specific membrane antigen (CH65)

One such chondrocyte surface molecule is serine and asparagine-rich peptide, with a molecular weight of 65 kDa (CH65). The CH65 antigen was detected in about 60-70% of sera of RA patients [71]. It was a chondrocyte-specific constitutively expressed autoantigen. CH65 displayed high homology with cytokeratins and the HSP65 – small heat shock protein that functions as a chaperone protein probably maintaining denatured proteins in a folding-competent state [61, 72, 73]. PBMC of 50% of the RA patients exhibited strong proliferative response to CH65 in culture, contrary to PBMC of healthy donors. Moreover, CH65-stimulated RA PBMC produced interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin-6 (IL-6). These results suggested that CH65 could act as a potential RA autoantigen [74].

Human cartilage glycoprotein-39 (HC gp-39)

Chondrocytes and synovial cells derived from RA patients secrete a 39 kDa glycoprotein named HC gp-39, which belongs to chitinase protein family, but does not possess activity against chitinase substrates. This autoantigen, which may act as an autoantigen in the immune response that develops in patients with RA, was rarely expressed in healthy people [75]. HC gp-39 is one of the major proteins produced and secreted by cultured articular chondrocytes and synovial fibroblasts. But neither the protein nor mRNA for HC gp-39 was detectable in normal newborn or adult human articular cartilage, while mRNA for this protein was detected both in synovial specimens and in cartilage obtained from patients with RA. It can be related to a response of the cells of articular cartilage and synovium to an inflammatory process [76]. It was also shown that the level of HC gp-39 was significantly higher in the plasma of RA patients than in the OA, SLE (systemic lupus erythematosus), or IBD (inflammatory bowel disease) patients and healthy control individuals. Moreover, the level of HC gp-39 in plasma of RA patients positively correlated with ESR (erythrocyte sedimentation rate) and IgM rheumatoid factor level. The level of HC gp-39 in the plasma of patients with OA, SLE, and IBD was also higher than in healthy controls, but no correlation was found with the disease activity score. Probably, the increased level of HC gp-39 did not only reflect the degree of joint disease but was related to inflammation and tissue degradation [77]. It has been demonstrated that the HC-gp mRNA level was higher in PBMC and synovium cells of RA patients than OA, SpA (spondyloarthropathy), SLE patients, and healthy control individuals. There were no significant differences among OA, SpA, SLE, and healthy controls [78]. To explain the role of HC gp-39 in matrix turnover and degradation the human skin fibroblasts and articular chondrocytes were stimulated with IL-1 and TNF in the presence of HC gp-39. It was shown that HC gp-39 suppressed the cytokine-induced production and secretion of metalloproteinases MMP1, MMP3, MMP13, and interleukine-8 (IL-8). Thus, HC gp-39 may play a role in limiting the catabolic effect of pro-inflammatory cytokines that are responsible for pathological loss of ECM, particularly that of cartilage in inflammatory and degenerative arthritis [79]. The other data also indicated that HC gp-39 participated in maintaining the balance between pro-inflammatory and regulatory responses in humans. PBMC from healthy individuals reacted against HC gp-39 with the production of interleukin-10 (IL-10) but not IFN-γ. Moreover, $CD4^+$ T cell lines directed against HC gp-39 expressed CD25, glucocorticoid-induced tumour necrosis factor receptor (GITR), and forkhead box protein 3 (Foxp3) molecules (markers of regulatory T cells – Treg) and were capable of suppressing antigen-specific recall T cell responses. In contrast, the HC gp-39-directed immune response in 50% of RA patients exhibited polarisation toward a pro-inflam-

matory Th1 phenotype and was not as effective in suppressing antigen-specific recall responses. These findings indicated that the presence of HC gp-39-specific immune responses in healthy individuals might have an inhibitory effect on inflammatory responses. Furthermore, these data suggested that HC gp-39-directed immune response in RA patients had shifted from an anti-inflammatory to a pro-inflammatory phenotype [80]. In the early phase of the GPI (glucose-6-phosphate isomerase)-induced arthritis in mice, the serum level of HC gp-39 increased and its mRNA level increased in splenic $CD4^+CD25^+$ $Foxp3^+$ regulatory T cells, but not in Th1, Th2, or Th17 cells. Furthermore, the addition of recombinant HC gp-39 caused the suppression of T-cell proliferation, and IFN-γ and interleukin-17 (IL-17) production. It suggested that HC gp-39 in $CD4^+$ T cells might play a regulatory role in RA [81]. Studies of the presentation of immunodominant epitope of HC gp-39 by shared epitope-positive synovial dendritic cells indicated that this presentation was associated with characteristic histologic features of follicular synovitis and is highly specific for RA [82]. Autoantibodies against HC gp-39 were detected only in the sera of 8% of RA patients, but not in samples from SLE patients or healthy donors [83].

Rheumatoid arthritis-associated antigen 47 (RA-A47)

The serum levels of antibodies directed against numerous proteins were relatively high in RA patients, whose joints exhibited a high degree of erosion, but the antibodies against 47 kDa protein appeared at an early state of disease and were continuously produced at high levels relative to controls. This protein was isolated, characterised, and identified as a colligin2/HSP47 (heat shock protein 47), a collagen binding protein, and named RA-A47 – rheumatoid arthritis-associated antigen [84]. It belongs to the serpin family and serves as a chaperone protein for collagen type I, II, III, IV, and V and is localised exclusively in endoplasmic reticulum (ER) [85]. HSP47 binds to the procollagen after it enters the ER, forms triple helix, and proceeds to the Golgi apparatus, where HSP47 dissociates from it and procollagen is secreted. This protein is involved in processing and secretion of collagens with correct conformation and prevents the secretion of abnormal procollagens [86]. The expression of HSP47 has been shown to be raised together with the expression of COL2A1 (type II collagen) gene upon stimulation with transforming growth factor-β (TGF-β). Pro-inflammatory cytokines – TNF, interferon-β (IFN-β), and IL-6, however, down-regulate the expression of HSP47, without repression of COL2A1. It was also shown that surface type II collagen disappeared in chondrocytes after TNF stimulation. Probably the decreased expression of HSP47 might inhibit type II collagen secretion and its accumulation inside the cell. TNF stimulated also the synthesis of iNOS (inducible nitric oxide [NO] synthase) and MMP9. These observations demonstrated that under the influence of TNF HSP47 was down-regulated, and secreted type II collagen was not accumulated inside the cell, while ECM was degraded by MMPs and iNOS; NO has been demonstrated to activate nuclear factor-κB (NF-κB), an important transcription factor that promotes the inflammatory response [87]. The localisation of HSP47 was also changed, to the surface or outside of the cells, and this change was probably responsible for the recognition of HSP47 as an RA-A47 – autoantigen in RA [88]. The treatment of the cells with ra-a47-specific anti-sense oligonucleotide resulted in down-regulation of total RA-A47 expression, and its increased presence on the cell surface. The cell surface expression of CD9 (a β1 integrin-associated transmembrane protein involved in cell adhesion and motility) was also enhanced. CD9 was colocalised on the cell surface with RA-A47. Furthermore, the FITC-labelled annexin V was bound to the cell surface, and active forms of caspase 9 and 7 were detected. Thus, the down-regulation of RA-A47, a chaperone protein, might induce apoptosis. The surface-exposed RA-A47 induced autoantibodies production and inflammatory reactions in autoimmune diseases, e.g. RA [89].

Hyaluronan binding adhesion molecule CD44

Articular chondrocytes expressed the CD44, a multifunctional adhesion molecule that binds to hyaluronan (HA), type I collagen, and fibronectin. CD44 receptors are broadly distributed, and their binding evokes numerous responses. These include cell adhesion, cell migration, induction (or at least support) of haematopoietic differentiation, changes in other cell adhesion mechanisms, and interaction with cell activation signals. This diversity of responses indicates that downstream events following ligand binding by CD44 can be different depending on the cell type and on the environment of that cell. In mature lymphocytes, CD44 is up-regulated in response to antigenic stimuli and may participate in the effector stage of immunological responses. CD44 ligand-binding function on lymphocytes is strictly regulated, such that most CD44-expressing cells do not constitutively bind ligand. Ligand-binding function may be activated as a result of differentiation, inside-out signalling, and/or extracellular stimuli. CD44 is not a single molecule, but instead a diverse family of molecules generated by alternate splicing of multiple exons of a single gene and by different post-translational modifications in different cell types. It is not yet clear how these modifications influence ligand-binding function [90]. CD44 receptors expressed on chondrocytes probably allow them to detect changes in matrix composition [91]. Thus, CD44 receptors play a critical role in maintaining cartilage homeostasis. Changes in interactions, either experimentally induced or detected in OA and RA, had profound effects on cartilage metabolism. The number of CD44-positive articular chondrocytes in RA was significantly higher than in OA, but the CD44 expression

displayed a more distinct zonal variation in OA than in normal articular cartilage [92]. It has been demonstrated that anti-CD44 monoclonal antibody reacted with all CD44 isoforms and markedly reduced the inflammatory activity of arthritis induced by collagen in mice [93]. Therefore, up-regulation of CD44 on articular chondrocytes in RA and changes in expression of this molecule on OA chondrocytes may play a significant role in cartilage degeneration [94, 95].

Thymocyte antigen-1 (Thy-1) – CD90

A new cell surface molecule, Thy-1 (thymocyte antigen-1) or CD90, expressed by human articular chondrocytes and synovial fibroblasts has recently been identified. This molecule is a heavily N-glycosylated, 25-37 kDa GPI (glycosylphosphatidylinositol)-anchored protein discovered as a thymocyte antigen. It regulates cell adhesion, migration, differentiation, and survival [96]. OA cartilage showed a higher expression of CD90 than normal tissue. Additionally, is has been shown that in vitro stimulation with pro-inflammatory cytokine IL-1β up-regulated its expression in the cartilage. These results suggested that Thy-1 might be a potential biomarker for cartilage pathogenesis, degradation, and metabolic turnover [97, 98]. Fibroblasts positive for CD90 were enriched in the synovium of RA patients [99], but only a minority of RA articular chondrocytes displayed a moderate CD90 expression [98].

Signal transducer – CD24

The next detected on the surface of chondrocytes surface glycoprotein anchored via GPI link to the cell was CD24. This protein contributes to a wide range of downstream signalling networks and is crucial in cell differentiation. High expression of CD24 molecule was observed on juvenile chondrocytes, which demonstrate higher cell proliferation rate and extracellular matrix production as compared to the adult chondrocytes, which exhibit only low expression of CD24. The loss of CD24 in adult chondrocytes led to an increase of NFκB activation and increased inflammatory and catabolic gene expression both in the presence and in the absence of IL-1β. Thus, CD24 is the regulator of inflammatory response that is altered during development and aging. Since inflammaging is associated with many forms of age-related pathological conditions and age is a risk factor in RA and OA, the restoration of the juvenile expression of CD24 on articular chondrocytes would be one of the targets of future therapy [100].

Lymphocyte function-associated antigen-3 (LFA-3) – CD58

The next antigen strongly expressed on some RA, moderately on OA chondrocytes, and not detected on normal articular chondrocytes is CD58. It is a cell adhesion molecule expressed on professional APCs, particularly monocytes/macrophages [98]. The ligand for CD58 is CD2, an adhesion molecule present on $CD4^+$ and $CD8^+$ T lymphocytes and NK cells. The CD2/CD58 binding is involved in most T-cell interactions with the other cells and in T-cell activation. Moreover, the binding of CD2/CD58 has significant importance in autoimmune diseases such as RA because it plays a crucial role in lymphocyte recruitment to the inflammatory sites [101, 102]. The levels of soluble form of CD58 (sCD58) were found to be significantly reduced in sera and synovial fluid of RA patients in relation to healthy donors and patients with SpA. The reduction of serum sCD58 correlated significantly with clinical and laboratory parameters of disease. Because locally released sCD58 blocks CD2/CB58 interaction, insufficient amounts of sCD58 in synovitis might result in T-cell accumulation and perpetuation of inflammation [103].

Type I transmembrane protein Tmp21

Osiecka-Iwan *et al.* [49, 104] found that chondrocyte-associated antigen, sialylated form of transmembrane protein Tmp21, was expressed on the surface of rat chondrocytes from articular-epiphyseal complexes. Tmp21 belongs to the p24 protein family. These proteins mainly participate in the traffic between the ER and Golgi complex, but in some cells they appear also in membranes of secretory vacuoles and on cell surfaces. Sialylated Tmp21 decreased in cultured chondrocytes concomitantly with the decline of collagen type II and aggrecan and the rise of collagen type I and versican expression. Moreover, its expression returned in chondrocyte re-cultured in three-dimensional cell culture together with the expression of collagen type II and aggrecan [105]. Because the sialylated form of Tmp21 has not been detected in other tissues, Tmp21 with its sialylated oligosaccharide moiety could be a chondrocyte differentiation antigen [49]. Because rat chondrocytes transplanted intramuscularly to rabbit evoked the production of antibodies directed against sialylated form of Tmp21 present on chondrocytes, it could be the target of further investigations on the role of this molecule in autoimmune diseases, such as RA.

Immunosuppressive and immunomodulatory properties of chondrocytes

Although chondrocytes express MHC class I and class II molecules [7], more recent observations suggest that they can also exert immunosuppressive and immunomodulatory effects on immunocompetent cells. In 1992 Jobanputra *et al.* [106] demonstrated that non-stimulated isolated human articular chondrocytes constitutively expressed MHC class I, MHC class II, and ICAM molecules and were able to substitute monocytes in triggering T-cell proliferation to mitogenic polyclonal stimuli, such

as phytohaemagglutinin A (PHA), they did not trigger, an efficient allogeneic immune response. Furthermore, IFN-γ and IL-1β treatment enhanced expression of MHC class II molecules and ICAM, but chondrocytes still failed to stimulate allogeneic PBMC. Contrary to previous authors, Adkinson *et al.* [18] found that isolated human articular chondrocytes did not constitutively express MHC class II molecules. Moreover, they did not express B7-1 (CD80) and B7-2 (CD86) molecules that provide a co-stimulatory signal necessary for T-cell activation, and suppressed, in a contact-dependent manner, proliferation of activated T cells. This suppression was associated with the expression of multiple negative regulators of immune responses. Adkinson *et al.* [18] detected that chondrocytes expressed other members of B7 peripheral membrane protein family, responsible for the inhibition of T-cell proliferation: programmed death-ligand 1 (PD-L1, B7-H1, CD274), programmed death-ligand 2 (PD-L2, B7-DC, CD273), B7-H2 (also called inducible co-stimulator-ligand – ICOS-ligand, CD275), B7-H3 (CD276), and V-domain Ig suppressor of T cell activation (VISTA, B7-H4). B7 molecules are membrane proteins found on activated antigen-presenting cells (APC), which bind to lymphocyte receptors CD28 or CD152 (cytotoxic T-lymphocyte-associated protein 4 – CTLA-4) and send a co-stimulatory or a co-inhibitory signal to enhance or decrease the activity of a MHC-TCR signal between the APC and the T cell [107]. Adkinson *et al.* [17] found that human chondrocytes expressed chondromodulin-I and indoleamine 2,3-dioxygenase (IDO). Chondromodulin-I is a cysteine-rich transmembrane protein originally purified from bovine epiphyseal cartilage that promotes chondrocyte proliferation [108, 109]. Mature protein can impact T-cell development or function; thus, chondromodulin-I expression by chondrocytes may directly disrupt T-cell function. IDO is the first and rate-limiting enzyme of tryptophan catabolism through the kynurenine pathway and has been implicated in immune modulation through its ability to limit T-cell function and engagement of mechanisms of immune tolerance [110]. The expression of mRNA for IDO was found also in human juvenile articular chondrocytes after IFN-γ treatment [14]. Similarly to Adkinson *et al.* [18], Lim *et al.* [14] found that isolated human juvenile articular chondrocytes expressed only MHC class I but not MHC class II and co-stimulatory molecules: B7-1 and B7-2. Also, these authors [14] observed that human chondrocytes expressed low levels of mRNA co-inhibitory molecule PD-L2. IFN-γ treatment of chondrocytes up-regulated the expression of PD-L2 and induced the expression of MHC class II molecules and co-inhibitory PD-L1 [14].

Some recent studies have shown that although articular chondrocytes expressed MHC class I molecules [14, 18], they did not stimulate alloantigen specific T-cell proliferation, but rather suppressed alloreactive T cells. This suppression was presumably mediated through the cell-to-cell contact with involvement of PD-L1 and PD-L2. Blocking of PD-L1 and/or PD-L2 with specific neutralising antibodies led to the restoration of alloreactive T-cell proliferation [14]. PD-L1 and PD-L2 probably promote self-tolerance and play a major role in suppressing the immune system during pregnancy, tissue allografts rejection, or autoimmune diseases. The binding of PD-L1 and PD-L2 to PD-1 transmits an inhibitory signal that reduces the proliferation of T cells and can also induce apoptosis by the regulation of the gene Bcl-2 expression [111, 112].

Similarly to previous authors, Lohan *et al.* [19] found that freshly isolated rat articular chondrocytes expressed low levels of MHC class I and negligible levels of MHC class II, CD80, and CD86 molecules. This expression was altered under the influence of oxygen tension and inflammatory cytokines (IFN-γ and TNF), but both primary and cytokine-stimulated cultured chondrocytes suppressed allogeneic T-cell proliferation in mixed lymphocyte/chondrocyte reaction. This inhibition could be dependent on decreased NO production [19]. NO probably represents an additional co-stimulatory signal for the induction of T cells [113]. Chondrocytes also have the ability to modulate inflammatory macrophage activity. The co-culture of macrophages and chondrocytes caused down-regulation of MHC class II molecules on lipopolysaccharide (LPS) or IFN-γ-stimulated macrophages [19]. Pereira *et al.* [13] demonstrated that although human articular chondrocytes expressed a low level of MHC class I and II molecules, they markedly inhibited T-lymphocyte proliferation to antigen-dependent and -independent proliferative stimuli. Moreover, chondrocytes inhibited the differentiation of peripheral blood monocytes to professional antigen-presenting cells [13].

Abbe *et al.* [21] found that OA chondrocytes have similar surface antigens as those from the healthy donors. Almost all studied cells expressed MHC class I molecules. Only 1 to 2% of chondrocytes expressed MHC class II molecules and less than 1% co-stimulatory molecules CD80 and CD86. OA chondrocytes inhibited proliferation of activated $CD4^+$ T cells *in vitro* via cell-cell contact and failed to elicit allogeneic $CD8^+$ T-cell reaction [21]. They also inhibited proliferation of lymphocytes stimulated by allogeneic antigens. This effect was observed even if recombinant IL-2 was added to the lymphocyte-chondrocyte culture. Thus, human articular chondrocytes could induce IL-2 non-responsiveness of allogeneic lymphocytes [114].

Conclusions

Therefore, although the numerous data on chondrocyte expression of class I and II MHC molecules and co-stimulatory and co-inhibitory surface molecules have been collected, it should be stressed that many of them were obtained as the results of in vitro studies and may not be directly applicable to the in vivo situation. Therefore, it is

probably unavoidable to verify them in clinical application and/or experiments with animal involvement.

The authors declare no conflict of interest.

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