EXPRESSION OF CD98 IN NORMAL AND OSTEOARTHRITIC SYNOVIAL TISSUES

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ÖZET

Giriş: Yüzey antijenlerince düzenlenen hücreler arası iletişim, sinovyal dokunun bütünlüğü için büyük önem taşır. Kronik inflamasyonla seyreden osteoartritte sinovyuma geçen lökositler birçok immünopatolojik olayı başlatır. CD 98, uyarılmış hücrelerde eksprese olduğu bilinen, bir tip II zar glukoproteinidir. Bu çalışmada CD 98'in osteoartritli insan sinovyumundaki ekpresyonunun normal ile karşılaştırılmalı olarak incelenmesi amaçlanmıştır.

Hastalar ve Yöntem: Bu çalışmada 10 adet osteoartritli hasta ve 6 adet normal olguya ait sinovyal doku örneği immünohistolojik olarak CD 98 ekspresyonu için değerlendirildi. Osteoartritli hasta örnekleri total eklem artroplastisi, kontrol örnekleri ise tanı amaçlı artroskopi uygulanan olgulardan elde edildi. Tip 2 sinovyositlerin ayrımı, asit fosfataz yöntemiyle gerçekleştirildi.

Sonuçlar: Normal sinovyum örneklerinde CD 98 tüm sinovyosit katlarında ve subsinovyal doku elemanlarının bazılarında eksprese oldu. Osteoartritli örneklerde tip A sinovyositlerde artmış ekspresyon saptandı.

Tartışma: Bu hücrelerin hızla aktive olarak osteoartrit patogenezinde, lenfosit infiltrasyonundan bağımsız olarak rol alabilecekleri düşünüldü.

Anahtar Kelimeler: *CD98, 4F2, Osteoartrit, Sinovyum, Immunhistoloji.*

NORMAL VE OSTEOARTRİTLİ SİNOVİYAL DOKULARDA CD 98'İN EKSPRESYONU

SUMMARY

Introduction: Intercellular communication mediated by cell surface antigens is important in the maintenance of synovial tissue (ST) integrity. Chronic inflammation is a common feature of osteoarthritis (OA) and leukocytes invade the ST initiating a number of immunopathological processes. CD98 is a type II membrane glycoprotein which is known to be strongly expressed on activated cells, cell lines and malignant or transformed cells.

Patients and Methods: The synovial lining layer and the underlying subsynovial tissue of 10 osteoarthritic patients and 6 normal individuals were evaluated immunohistologically for CD98 expression. STs were obtained from OA patients undergoing total joint arthroplasty and during diagnostic arthroscopy in the controls. Prepared sections were screened using an indirect immunoperoxidase method. Acid phosphatase staining was performed to identify the type A synoviocytes.

Results: CD98 was positive in the synoviocyte layer in almost all the lining layer of the normal ST. There was also a positive reaction in some of the ST stromal elements. A significant difference in antigenic phenotype of synovial lining cells in OA with the above mentioned activation marker was mainly present on the type A synoviocytes.

Discussion: Type A synoviocytes presented increased expression of CD98, suggesting that they are rapidly and fully activated. The fact that their recruitment was independent of the degree of lymphocyte infiltration further emphasizes the possible central importance of synovial lining layer in OA. The potential role of chronic cellular activation in the over-expression of CD98 in OA and ST, deserves further attention.

Key Words: *CD98, 4F2, Osteoarthritis, Synovium, Immunohistology.*

INTRODUCTION

Osteoarthritis (OA) is the most prevalent acquired connective tissue disorder that affects the synovial joints. This disease involves all the tissues of the joint including the synovium. Clinically, the disease is characterized by joint pain, tenderness, limitation of movement and functional disability. Studies on OA pointed out multiple biological and biochemical synovial tissue factors that are involved in the etiopathogenesis of the disease[1].

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Although inflammation is present to some extent in the natural course of OA, it is usually episodic and appears often to be a response to crystal mineral complexes and joint debris[1]. Thus, synovium is often considered to be at a normal like state in several studies[2,3]. However, concomitant trauma will furthermore increase inflammation and may enhance the production of biologic mediator molecules. The relationship between the inflammatory response, and the initiator and mediator molecules in OA are the scopes of recent research studies[4,5]. Some of these initiator or mediator molecules are supposed to be the adhesion molecules[4-6].

CD98 is a type II glycoprotein that is strongly expressed in almost all activated cells, cell lines and malignant or transformed cells[7-10]. The tissue distribution of this antigen has not been studied in OA STs so far. The localization of CD98 in normal and OA STs is comperatively evaluated by immunohistology, and the possible role of this antigen on the synovial response of OA inflamation is discussed in this study.

MATERIALS AND METHODS

Synovial tissues were obtained from 10 OA patients, and 6 normal individuals undergoing diagnostic arthroscopy established the controls. The average age of the two group was 65.0 ± 8.3 and 31.8 ± 5.3 respectively. Eight of the patients were female and two were male in the study group. The control group consisted of two females and four males. Synovial tissues were obtained from the hip and/or knee joint in the study group and from the knee joint in the control group. Data on the sex, age, diagnosis and surgery is given in Table I. All patients of the study group were undergoing total joint replacement due to OA, and presented joint pain and range of motion limitation clinically. Radiological findings of these patients were in accordance with the disease.

Table I
Cases

	n	Sex	Age	Joint
Patients	10	8F, 8M	65.0 ± 8.3 (53.78)	hip, knee
Controls	6	2F, 4M	31.8 ± 5.3 (27.39)	hip, knee

Immunohistological Study

All specimens were immediately frozen in liquid nitrogen and stored at -30°C. Stainings were performed within two or three days following surgery. Cryostat sections (6-8 mm thick) were obtained on gelatin-coated slides and kept in humidity-free containers at room temperature.

Monoclonal Antibody : The monoclonal primary antibody used in this study was supplied from the "Leukocyte Typing VI Workshop Kobe, 1996"[11]. Tissue distribution of CD98 was examined with BU53 (IgG_{2a}, Hardie) using the indirect immunoperoxidase assay.

Staining Procedure : The indirect immunoperoxidase procedure used in this study has been described in details by Dijkstra et al.[12]. Sections were fixed in acetone for 10 minutes and air-dried for at least 30 minutes. Then, they were incubated with the primary antibodies for 60 minutes. After washing with 0.01 M PBS at pH = 7.4, the sections were covered with rabbit anti-mouse IgG peroxidase (Sigma, USA). The dilution of IgG in PBS was 1:200 and contained 0.2% bovine serum albumin (BSA) and 1% normal human serum. The slides were then stained for peroxidase acivity with 3.3'-diaminobenzidine-tetrahydrochloride (DAB, Sigma, USA) (0.5 mg/ml Tris-HCl buffer, pH:7.6, containing 0.01% H_2O_2). A counterstaining with haematoxylin was performed respectively. Control stainings were performed by omitting the initial primary antibody staining step and using a control mouse IgG.

Acid Phosphatase Staining Procedure : Following the secondary antibody and the DAB staining steps, a group of tissues were placed into the humidified chamber and incubated at 37°C for 45 minutes with acid phosphatase solution[13]. The specimens were then washed with PBS and counter-stained with haematoxylin.

Stained sections were examined by at least three blinded observer. In general, no major differences were found between the analyses of the observers. At least five 40x magnification areas were examined by sections and some of them were photographed using an Olympus BH2 microscope. All immunostaining of ST components were graded by frequency in a range of 0-100%; where 0% indicated no staining and 100% indicated that almost all cells were immunoreactive in high magnification. The results showed that all the specimens exhibited the same broad distribution pattern in each group and almost all of the same type of cells were immunoreactive for CD98 or not. Thus the data have not been scaled.

RESULTS

In the OA group, a strong positive reaction for CD98 was present in the round shaped macrophage-like cells (type A synoviocytes) located at the outermost layer compared to that

of the fibroblast-like spindle shaped cells (type B synoviocytes) present at the inner layer of the ST (Figure 1a, b). In addition to the morphological criteriae, type A synoviocytes were also identified by acid phosphatase staining where they appeared redish. The endothelial cells expressed CD98 at the vasculature of the subsynovial tissue but not the smooth muscle layer (Figure 1a, b). A positive reaction was also observed in some of the stromal fibroblasts and macrophages that were near the vessels (Figure 1a, b). The synovial lining layer was stained with mAb for CD98 in the control group (Figure 2a, b). Both type A and type B synoviocytes expressed uniformly the above mentioned molecule (Figure 2a, b). The endothelium of the small vessels were also immunoreactive with CD98 antibody, similar to the OA group (Figure 2a, b).

DISCUSSION

Synovium is known to have important immunological features and is the target tissue in the articular joint during inflammation. Synovium is considered to be at a normal like state in OA and its immunohistochemical aspects is compared to that of rheumatoid arthritis in many studies since the inflammation is episodic and mainly absent in this disease[2,3]. In fact, there is still some contraversy on the pathological criteria of the synovial involvement in osteoarthritis[1].

CD98 is a 125.000 M_r type II glycoprotein[14] composed of a 80.000 M_r glycosilated heavy chain (CD98 hc) and a 45 000 M_r non-glycosylated light chain (CD98 lc)[9]. It is strongly expressed in almost all activated cells, cell lines and malignant or

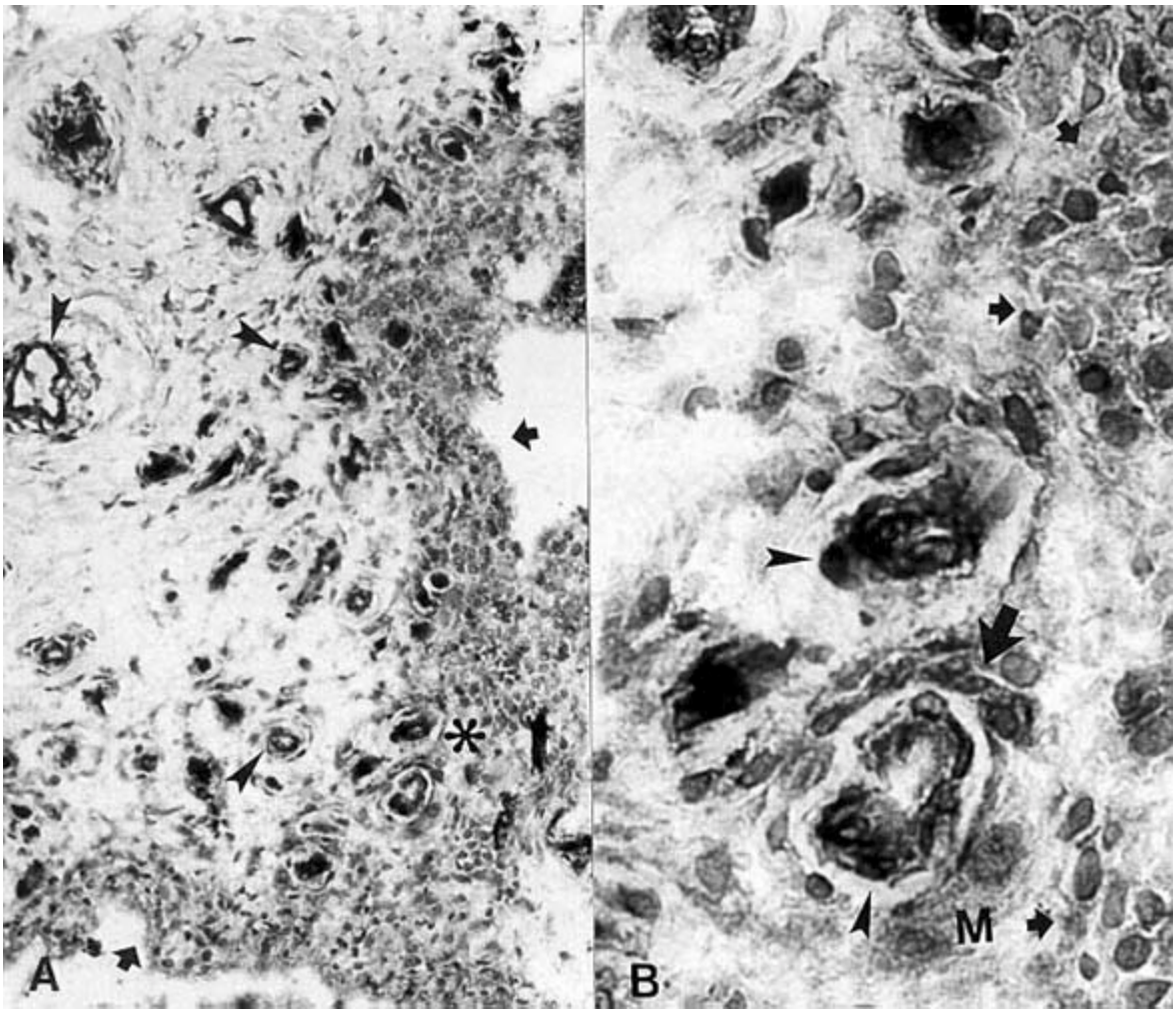

Figure 1: a) Indirect immunoperoxidase staining of frozen OA ST using mAb BU53 recognizing CD98. ST lining reactivity at the uppermost layer is indicated by arrows. Arrow head shows the positive stained vascular endothelium wthin the stroma. Round shaped macrophage-like synoviocytes reactivity is indicated by double arrow heads. b) Micrograph showing strong CD98 expression in the subsynovial macrophages (M) and vascular endothelium (arrowheads). Original magnification x 40, Haematoxylin.

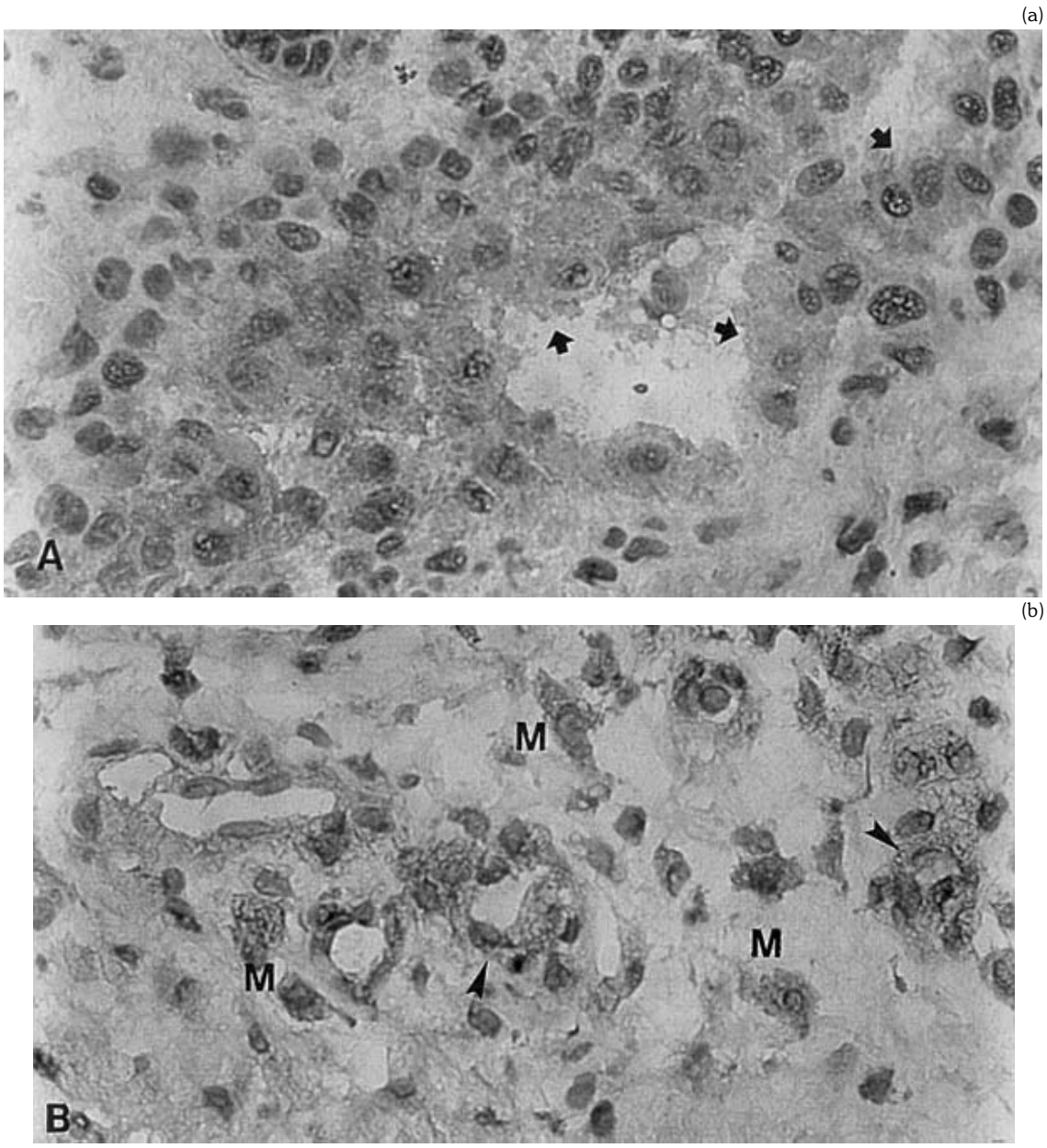

Figure 2: a) Indirect immunoperoxidase staining of frozen control ST for CD98, Expression is evident in all the layers of the lining (arrows) and the subsynovial vascular endothelium (arrowhead). b) The vascular endothelium (arrowheads), the fibroblast (big arrow)s surrounding the vessels and the macrophages (M) are immunoreactive with CD98 within the subsynovium. Original magnification x10, x40, Haematoxylin.

transformed cells. Low levels of this molecule are expressed on resting peripheral blood mononuclear cells[7,9]. The human and mouse CD98hc cDNAs are cloned and present limited sequence homology[15,16].

In this study, all the OA tissues presented significant differences compared to the control group for CD98 expression on the synoviocyte layer. The expression of this antigen remained almost constant at the subsynovial tissue in OA and control groups, whereas this expression apparently changed in the upper layers and especially in the type A synoviocytes in OA. The widespread expression of this antigen in normal tissues may suggest its indirect role on the maintenance of tissue integrity *via* some other molecules. The constant expression of this molecule both in OA and controls also suggests local endothelial activation by proinflammatory cytokines, however,

they do not necessarily regulate the level of expression. Mainly type A synoviocytes located at the uppermost lining layer presented increased expression of this early activation antigen in OA. This may suggest that these cells are rapidly activated.

The role of CD98 in cellular activation is unknown, however, several reports investigated the function of this molecule in T cell responses[7,9,16-18]. There is evidence that CD98 may regulate T cell activation distal to the cascade of initial signalling events. The functional effects of anti-CD98 mAb on T cell activation may or may not be related to the putative amino acid transport properties of the CD98hc[16,19-21]. Rheumatoid and reactive arthritic synovial fluids T cells were stained for activation markers including CD98[22-26]. Activated terminal effector T cells of suppressor/cytotoxic nature were rare and presented low CD98 expression. It is reported that the ability of synovial fluid lymphocytes to become activated does not change in RA as *in vitro* mitogen stimuation of these cells induced expression of all the activation markers including CD98, in spite of a low proliferative response[24]. Decreased expression of CD98 was reported in RA in several studies[22-26]. This may suggest the down regulation[24] or the prior activation[25] of lymphocytes in vivo.

No significant lymphocytic infiltration was noted in any of the cases. CD98, on the other hand, presented a broad distribution within the synovial tissue elements; mainly in the lining layer and vascular endothelium. The fact that the activation was independent of the degree of lymphocyte infiltration may further emphasize the possible central importance of the synovial lining layer in OA. The shift towards the superficially located type A synoviocytes may indicate a reactivation or down regulation of expression of activation antigens of these cells in OA. The potential role of this molecule on OA synovium may possibly be related to the other CD98 associated molecules[27,28]. The structural and the functional characterization of the light chain may also clarify the functional capacity of CD98 complex on the OA synovium[16].

ST may have important immunological features as a target tissue in the immunopathology of OA. The potential role of chronic cellular activation in the over-expression of CD98 in OA and ST, deserves further attention.

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REFERENCES

1. Gardner DL. Osteoarthrosis and allied disorders. In Gardner DL Ed. Pathological Basis of the Connective Tissue Disorders (1st ed). London: Rowland Ltd, 1992, 842-921.
2. Koch AE, Burrows JC, Haines GK. Immunolocalization of leukocyte and endothelial adhesion molecules in human rheumatoid and osteoarthritic synovial tissue. Lab Invest 1991; 64: 313-320.
3. Dolhain RJ, Haar NT, Hoefakker S, Tak P, Ley MD, Claassen E, Breedveld FC, Miltenburg AM. Increased expression of interferon (IFN) gamma together with IFN-gamma receptor in the rheumatoid synovial membrane compared with synovium of patients with osteoarthritis. Br J Rheumatol 1996; 35: 24-32.
4. Johnson BA, Haines GK, Harlow LA, Koch AE. Adhesion molecule expression in human synovial tissue. Arthritis Rheum 1993; 36: 137-146.
5. Szekanecz Z, Harlow GK, Harlow LA, Shah MR, Fong TW, Fu R, Lin SJW, Koch AE. Increased synovial expression of the adhesion molecules CD66a, CD66b, and CD31 in rheumatoid and osteoarthritis. Clin Immun Immunopathol 1995; 76: 180-186.
6. Ronday HK, Smits HH, van Muijen GNP. Difference in expression of the plasminogen activation system in synovial tissue of patients with rheumatoid arthritis and osteoarthritis. Br J Rheumatol 1996; 35: 416-423.
7. Hemler ME, Strominger JL. Characterization of the antigen recognized by the monoclonal antibody (4F2): Different molecular forms on human T and B lymphoblastoid cell lines. J Immunol 1982; 129: 623-628.
8. Parmacek MS, Karpinski BA, Gottesdiener KM, Craig BT, Leiden JM. Structure, expression and regulation of the murine 4F2 heavy chain. Nucleic Acid Res 1989; 17: 1915-1931.
9. Haynes BF, Hemler ME, Mann DL, Eisenbarth GS, Shelhamer J, Mostowski HS, Thomjas CA, Strominger JL, Fauci AS. Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. J Immunol 1981; 126: 1409-1414.
10. Fais S, Pallone F. Ability of human colonic epithelium to express the 4F2 antigen, the common acute lymphoblastic leukemia antigen, and the transferrin receptor. Gastroenterology 1989; 97: 1435-1441.
11. Dijkstra CD, Döpp EA, Joling P, Kraal G. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in rat recognized by monoclonal antibodies ED1, ED2 and ED3. J Immunol 1985; 54: 589-599.
12. Kishimoto T, von dem Borne AEG, Goyert SM, Kikutani H, Mason D, Miyasaka M, Moretta L, Okumura K, Springer T. Leukocyte Typing VI. New York: Garland Publishing Inc. 1997.
13. De Jong ASH. Mechanisms of metal-salt methods in enzyme cytochemistry with special reference to acid phosphatase. Histochem J 1982; 14: 1-33.
14. Teixeira S, Di Grandi S, Kuhn LC. Primary structure of the human 4F2 antigen heavy chain predicts a transmembrane protein with a cytoplasmic NH2 terminus J Biol Chem 1987; 262: 9574-9580.

15. Kuhn LC, Barbosa JA, Kamarck ME. An approach to the cloning of cell surface protein genes. Selection by cell sortinf of mouse L-cells that express HLA or 4F2 antigens after transformation with total human DNA. Molecular Biol and Medicine 1983; 1: 335-352.

16. Luis A, Diaz Jr PA, Endres J. CD98 workshop panel report. In: Kishimoto T, von dem Borne AEG, Goyert SM, Kikutani H, Mason D, Miyasaka M, Moretta L, Okumura K, Springer T. eds. Leucocyte Typing VI, New York: Garland Publishing Inc. 1997, 531-533.

17. Freidman AW, Diaz Jr LA, Moore S, Schaller J, Fox DA. The human 4F2 antigen: Evidence for criptic and noncryptic epitopes and for a role of 4F2 in human T lymphocyte activation. Cell Immunol 1994; 154: 253-263.

18. Diaz jr LA, Freidman AW, Jawanda JS. A functional role for the 4F2 antigen-CD98 in T-lymphocyte activation. *In* Schlosmann SF, Boumsel L, Gilks W, Harlan JM, Kishimoto T, Moromoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder TF, Todd RF. Eds. Leucocyte Typing V, Oxford, Oxford University press, 1995, 281-283.

19. Hediger MA, Kanai Y, Lee WS. Identification of a new family of proteins involved in amino acid transport. Soc Gen Physiol Ser 1992; 48: 301-314.

20. Fei YJ, Prasad PD, Leibach FH. The aminoacid transport system y+L induced in xenopus laevis oocytes by human choriocarcinoma cell (JAR) mRNA is functionally related to the heavy chain of the 4F2 cell surface antigen. Biochemistry 1995; 34: 8744-8751.

21. Broer S, Broer A, Hamprecht B. The 4F2 hc surface antigen is necessary for expression of system L-like neutral aminoacid-transport activity in C6-BU-1 rat glioma cells: evidence from expression studies in xenopus laevisoocytes. Biochem J 1995; 312 (Pt3): 863-870.

22. Goto M, Zvaifler NJ: Characterization of the natural killer-like lymphocytes in rheumatoid synovial fluid. J Immunol 1985; 134: 1483-1486.

23. Konttinen Y, Bergroth V, Nykanen P. Lymphocyte activation in rheumatoid arthritis synovial fluid in vivo. Scand J Immunol 1985; 22: 503-507.

24. Konttinen YT, Nordstrom D, Bergroth V. Cell-mediated immun response in the diseased joints in patients with reactive arthritis. Scand J Immunol 1986; 23: 685-691.

25. Smith MD, Roberts Thomson PJ. Lymphocyte surface marker expression in rheumatic diseases: evidence for prior activation of lymphoctes in vivo. Ann Rheum Dis 1990; 49: 81-87.

26. Hoy MD, O'Donnell JL, Hart DN. Dual CD45RA, CD45RO positive T-lymphocytes within rheumatoid arthritic joints. Pathology 1993; 25: 167-173.

27. Azzarone B, Suarez H, Mingari MC, Moretta L, Fauci AS. 4F2 monoclonal antibody recognizes a surface antigen on spread human fibroblasts of embryonic but not of adult origin. J Cell Biol 1984; 98: 1133-1137.

28. Bron C, Rousseaux M, Spiazzi AL. Structural homology between the human 4F2 antigen and a murine cell surface glycoprotein associated with lymphocyte activation. J Immunol 1986; 137: 397-399.