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Expression of a multidrug resistance gene in human rheumatoid synovium

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Abstract The objective of this study was to assess the expression of a multidrug resistance (MDR) phenotype, implicated in the cellular resistance of tumor to chemotherapy, in rheumatoid synovial membrane. Synovial membrane from 16 rheumatoid (RA) patients was studied. Six patients with osteoarthritis constituted the control group. The cell membrane expression of the glycoprotein Pgp 170, encoded by the MDR 1 gene, was determined by an immunoperoxidase technique using two different monoclonal antibodies (JSB 1, C219). The polymeras chain reaction (PCR) methods were used in parallel to detect the presence of the MDR 1 gene mRNA in the synovial cells. Pgp 170 was expressed on the cell membrane of five RA patients and MDR 1 cellular transcription was detected in one other RA patient. We did not observe any association between synovial glycoprotein expression and age, disease activity, and a specific treatment with a long-acting drug. However, MDR protein expression was associated with the successive treatment with more than three disease-modifying antirheumatic drugs (DMARDs). We conclused that the synovial membrane expresses a glycoprotein recognized by the antibodies JSB 1 and C219. The absence of concomitant MDR 1 transcription suggests the expression of an atypical MDR phenotype in the synovial membrane, distinct from the Pgp 170 encoded by the MDR 1 gene. The implications of the MDR phenotype and the resistance of RA to DMARDs is further discussed.

Key words Rheumatoid arthritis · Resistance to treatment · MDR gene

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Introduction

Several biological mechanisms of chemoresistance have been described in cancers. Selected cellular cross-resistance to structurally unrelated drugs is a consequence of MDR 1 gene expression [1]. This gene has been determined by the study of tumor cell lines selected by their resistance to the vinca alkaloids and doxorubicin [2]. The chemoresistance has been shown to be related to an increased efflux of the drug from the cells [3]. The multidrug-resistant cell lines have an amplified MDR 1 gene, and overexpress the encoded P glycoprotein (Pgp 170). This glycoprotein is an intracellular energy dependent transporter, and has considerable sequence homology with a number of bacterial transport proteins [3]. Pgp is not only expressed by malignant cells, but has also been identified in normal tissues with an excretory function, such as the proximal tubules of the kidney, the liver biliary ducts, and endothelial cells of the blood barrier, and lymphocytes [4, 5].

In rheumatoid arthritis (RA), progressive unresponsiveness to disease-modifying antirheumatic drugs (DMARDs) constitutes a major obstacle to the treatment of the disease. Cell lines of epithelial cells with acquired resistance to gold have been obtained by incubating the cells with gold. The resistance has been shown to be related to the induction of metallothionein, a protein that is able to bind gold [6]. Methotrexate (MTX) is not considered as a member of the MDR 1 family of drugs because the cell lines expressing Pgp 170 remain sensitive to MTX [1]. Cell line resistance to MTX has been shown to be related to an increase in the expression of the drug target, dihydrofolate reductase, by gene amplification [7]. However, the CCRF-CEM cells, which have been selected in vitro for their extreme resistance to MTX, express a glycoprotein recognized by the monoclonal antibody (mAb) C219, but Northern blotting has failed to demonstrate any overexpression of the MDR 1 genes [8]. These results suggest that the glycoprotein expressed on the MTX-resistant cells is different from the Pgp 170 encoded by the MDR 1 gene. Norris et al. have called the MTX

resistance in CCRF-CEM cells the atypical MDR phenotype [8].

To study the acquired resistance of RA to DMARDs, we investigated the expression of Pgp 170 by immunohistology on synovial tissue obtained from both RA patients and osteoarthritis patients. We also investigated the presence of the MDR 1 gene mRNA by polymerase chain reaction (PCR) in the same patients. Our results showed that synovial tissue expressed a Pgp recognized by the mAbs C 219 and JSB 1 in 31% of RA patients. However, we detected a transcription of the MDR 1 gene in only one RA patient. These results suggested that an atypical MDR phenotype may be expressed by the synovial cells in RA.

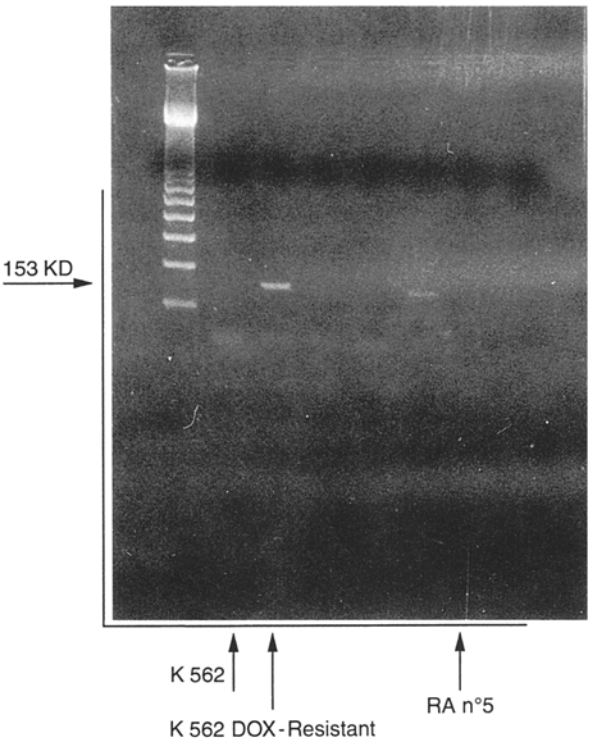

Fig. 1 Analysis of the presence of mRNA of MDR 1 gene in the synovial tissue of rheumatoid arthritis (RA) patients (*lanes 4–10*). *Lane 1* represents the migration of the marker, *lane 2*, the migration of the drug-sensitive human erythroleukemia cell line K 562, and *lane 3* the K 562 Dox-resistant cell line expressing MDR 1 gene

Patients and methods

Patients

Synovial membrane was obtained surgically from 16 patients (8 males and 8 females) with RA diagnosed according to the ARA revised criteria [9]. Their mean age was 56 years (range 30–79) and the mean disease duration was 9 years (range 1–12 years). Eight patients had active disease at the time of the study defined by the presence of at least three of the following criteria: ESR above 28 mm in the first hour (Westergren method), Ritchie index [10] above 10, more than six swollen joints, and morning stiffness for longer than 45 min. Rheumatoid factor was detected by nephelometry in nine of the patients. Synovial membrane was also obtained from six patients with osteoarthritis who constituted the control group. There were three men and three women in this group with a mean age of 58.6 years. Informed consent was obtained from all patients.

Immunohistochemistry

Consecutive 4-*M* sections of the frozen tissues were cut for immunostaining. The primary antibodies were (CD 3 Immunotech, Marseille, France) and two Mabs recognizing two different epitopes of Pgp 170, JSB 1 (Sanbio, Uden, Netherlands) and C 219 (Centocor Inc, Malvern, Pa.). An isotope matching Mab IgG 2 a was used as a control in each experiment. After washing with phosphat-buffered saline (PBS), a peroxidase-stained rabbit anti-mouse antibody was applied for 60 min.

Cell line and tissues

The drug-sensitive K 562 human erythroleukemia cell line was purchased from American Type Culture Collection, Rocheville, USA and routinely cultured in RPMI medium with 10% fetal calf serum. The TK 562/DOX-resistant cell line was a kind gift from Dr. Robert, Bordeaux, France. Synovial tissue was snap frozen in liquid nitrogen within 1 h of surgery and pulverized while frozen before RNA extraction.

RNA isolation and cDNA synthesis

After removal from –70 °C, the tissue was minced in PBS solution and homogenized in an Ultra-Turrax T 25 (IKA-Labortechnik Germany). The total RNA was extracted using the guanidium thiocyanate-phenol-chloroform method [11]. The RNA yield was measured by a spectrophotometer. Reverse transcription of 0.5 µg RNA with 25 pmol of random hexadeoxynucleotide (Pharmacia) was performed in a 30-µl solution using 300 U M-MLV reverse transcriptase, 5 m*M* DTT, 1 X RT buffer (Gibco BRL), 60 U RNAsin (Promega), and 1 m*M* dNTP (Pharmacia). After 1 h at 42 °, the sample was then heated to 95 °C for 5 min to stop the reverse transcription.

PCR amplification

To evaluate the quality of the cDNA, we first carried out a PCR amplification on the β-globulin gene using the following primers: primer 1: 5′-GCAACCTCAAACAGACACCPCR-3′, nucleotide position 135–153; primer 2: 5′-CTCAAAGAACCTCTGGGTCC-3′, nucleotide position 396–415 [12]. One microliter of cDNA obtained previously was added to a solution containing 100 pmol of each primer, 1 X Taq buffer, 2 m*M* $MgCl_2$, 2.5 U Taq polymerase (Premega), and 0.25 m*M* dNTP in a final volume of 100 µl. The optimum conditions for using a 9600 Paerkin Elmer/Cetus Thermal Cycle were: denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. A total of 35 cycles were performed followed by a final extension step at 72 °C for 10 min. The amplification generated a 151-bp product as expected.

The sense MDR 1 primer 1, 5′-ATATCAGCAAGCCACATCAT-3′, corresponds to MDR 1 cDNA 3007–3026. Primer 2, 5′-GAAGCACTGGGATGTCCGCT-3′, corresponds to the antisense strand of MDR1 cDNA sequence 3141–3160 [13]. The reaction was carried out with 1 µl cDNA in a total volume of 100 µl containing 100 pmol of each primer, 1 X PCR buffer, 3 m*M* $MgCl_2$, 2.5 Taq polymerase, and 0.8 m*M* dNTP. Each cycle included a 20-s denaturation step at 94 °C, a 30-s annealing step at 58 °C, 30 s of extension at 72 °C, followed by a final 7-min elongation at 72 °C. A total of 35 cycles were performed. Samples were then heated at 95 °C for 10 min. The PCR product was separated on a 2% agarose (Nusieve, Tebu) gel electrophoresis. All the necessary precautions against contamination of the PCR were rigorously observed.

Table 1 Expression of membrane Pgp 170 by immunohistology and of MDR 1 gene mRNA amplification on synovial tissue in 16 rheumatoid arthritis patients. The two last columns represent the expression of membrane glycoprotein by immunohistology with the mAbs C219 and JSB 1 (*RF+* rheumatoid factor >40 UI on nephelometry, *Nb DMARD* number of previous long-acting drugs used in treatment, *DMARD* disease-modifying antirheumatic drug, *MTX* methotrexate, *DP* D-penicillamine, *SLZ* salazopyrine, *Gold* gold salts, *PCR MDR-1* presence of MDR 1 gene mRNA determined by polymerase chain reaction)

Patients	Sex	Age (years)	Active disease	RF	Nb DMARD	DMARD	PCR MDR 1	C219	JSB 1
1	M	30	yes	+	5	steroids	–	+	+
2	M	34	yes	+	4	MTX	–	–	+
3	F	39	no	+	3	SLZ	–	–	+
4	M	64	yes	–	3	SLZ	–	+	+
5	F	51	no	+	3	GOLD	+	–	–
6	H	58	no	+	3	SLZ	–	+	+
7	M	71	yes	+	4	MTX	–	–	–
8	F	57	yes	–	2	MTX	–	–	–
9	F	67	no	+	2	DP	–	–	–
10	F	63	no	–	1	SLZ	–	–	–
11	F	76	no	–	2	steroids	–	–	–
12	M	63	yes	–	2	MTX	–	–	–
13	M	62	no	–	2	GOLD	–	–	–
14	M	79	no	+	4	steroids	–	–	–
15	M	40	no	–	2	DP	–	–	–
16	F	56	yes	+	4	MTX	–	–	–

The specificity of the PCR was attested by the negative results obtained with the drug-sensitive K 562 cell line, where no expression of the MDR1 gene was found in six independent experiments. The sensitivity of the PCR was assessed by performing the same protocol with the cDNA extracted from the K 562/DOX-resistant cell line constitutionally expressing the MDR 1 gene at different dilutions (Fig. 1). The PCR was conducted with, respectively, 1 µg cDNA, 0.1 µg cDNA, 0.01 µg cDNA, 0.001 µg cDNA, and 0.0001 µg cDNA. The sensibility of the method was estimated to be the detection of one MDR 1-positive cell out of 10,000 negative cells.

Statistics

The statistical significance between age, disease duration, the number of DMARDs used successively to treat the patients and the glycoprotein expression was studied by the Fisher exact test.

Results

Immunohistology

A membrane glycoprotein was expressed in the synovial tissue of 5 of the 16 RA patients (31.25%), whereas only 1 patient with osteoarthritis had a weak expression in the synovial membrane ($P<0.05$). Up to 30% of the cells in the RA samples expressed the glycoprotein on the cell surface. Staining with JSB 1 appeared to be more sensitive, and two RA patients (nos. 1 and 4) were C219 negative but JSB 1 positive.

PCR

Using the PCR method, we demonstrated the presence of mRNA of the MDR 1 gene in the synovial tissue of one of the RA patients (no. 5), but in none of the control group (Fig. 1). This patient had no detectable membrane expression of Pgp 170 by immunohistology.

Clinical associations

As shown in Table 1, the five RA patients with positive staining for the glycoprotein by JSB 1 or C 219 and patient no. 5 who had MDR 1 transcription had been treated successively with three different DMARDs, and this was statistically different from the remaining RA patients ($P<0.01$). Patient no. 1 developed proteinuria with gold salts and then with D-penicillamine (DP), without joint improvement. He was then treated with Salazopyrin, but this drug was withdrawn because of thrombopenia. MTX was then introduced, but an allergic pneumonitis led to the withdrawal of MTX. This patients with severe RA is currently being treated with steroids alone. Patient no. 2 did not respond to gold salts or chloroquine. After a transient response to DP, he presented with a flare-up of the disease, and MTX was initiated at 10 mg per week. Patients 3 and 6 were not improved by gold or DP, and responded to Salazopyrin. Patient no. 4 improved with gold salts, but the treatment was withdrawn because of proteinuria. A clinical response was obtained with Salazopyrin. Patient no. 5 was refractory to DP and hydxychloroquine, but responded well to gold salts. No associations was found between age, disease duration, disease activity, or the particular treatment at the time the samples were obtained.

Discussion

The overexpression of the MDR 1 gene was initially described in human tumor cell lines resistant to chemotherapy [1]. This gene encodes a membrane glycoprotein, Pgp 170 [3]. This protein is a cytosolic transporter that binds the drug and actively extrudes it out of the cell. Tumors such as carcinoma of the liver, the kidney or the adrenal gland are intrinsically resistant cancers and primitively express the MDR 1 gene amplification will be induced by the cytotoxic drug, and a clinical correlation has

been demonstrated between the unresponsiveness of the patients to chemotherapy and the presence of mRNA of the MDR 1 gene in the tumor [15]. Expression of the MDR 1 gene is not limited to tumor, and normal tissues express Pgp 170 [4]. The liver, the renal tubule, the endothelial cells of the brain barrier, and blood lymphocytes express the membrane protein Pgp 170 [5]. The physiologic role of the MDR 1 gene in normal tissues is not known, but excretion of toxic metabolites has been suggested [16].

We showed that a glycoprotein recognized by the mAbs C 219 and JSB 1 was expressed on the synovial membrane of RA patients treated with distinct DMARDs and also in one patient with osteoarthritis. In RA, acquired unresponsiveness to DMARDs remains a critical problem. Epithelial cell lines resistant to gold have been induced after incubation with gold salts. These cells express metallothionein, a protein that binds gold salts [6]. Moreover, an increase in the capacity of glutathion to conjugate gold might explain the resistance to the treatment. When the drug is conjugated, a cytosolic transporter actively pumps the gold conjugate out of the cell. It has been suggested that Pgp 170 might be the glutathion conjugate pump [17]. In multiple myeloma, response to chemotherapy has been shown to be regulated both by the MDR gene and by glutathion transferase genes [18]. More recently, MTX has been shown to induce the expression of dihydrofolate reductase in vitro, but the cells expressing the MDR 1 gene remain sensitive to MTX [7]. The long-acting drugs used in RA, including MTX, are not known to induce MDR 1 transcription. However, MTX-resistant cell lines have been shown to express an atypical MDR phenotype, distinct from Pgp 170 and recognized by the mAb C 219. The CCRF-CEM cells selected in vitro for their extreme resistance to MTX express a glycoprotein recognized by the mAb C 219, but Northern blotting has failed to demonstrate any overexpression of the MDR 1 genes [8, 19]. These results suggest that the glycoprotein expressed on the MTX-resistant cells is different from the Pgp 170 encoded by the MDR 1 gene. Norris et al. have called the MTX resistance in CCRF-CEM cells the atypical MDR phenotype [8]. In our study, the expression of glycoprotein in the absence of MDR 1 transcription suggested a similar atypical MDR phenotype in the synovial membrane, distinct from Pgp 170. A positive expression of MDR protein was associated with the use of more than three DMARDs, but not with a specific drug. An increased number of patients will be required to assess the association between DMARD resistance in RA and the expression of the atypical MDR phenotype in the synovial membrane. Cyclosporin has been shown to regulate MDR gene expression [20]. This drug is an efficient treatment of severe RA resistant to MTX and an association of cyclosporin with MTX has been proposed [21]. A possible mechanism of action of cyclosporin in RA resistant to MTX might be the modulation of the MDR gene.

These results suggested that atypical MDR genes are actively expressed in rheumatoid synovium. One hypothesis is that the acquired DMARD resistance in RA might be associated with the MDR phenotype. Further studies are clearly needed to assess this hypothesis.

References

1. Flier JS, Underhill LH (1987) Multiple drug resistance in human cancer. N Engl J Med 316:1388–1393
2. Kartner N, Riordan JR, Ling V (1983) Cell surface P glycoprotein associated with multiple-drug resistance in mammalian cell lines. Science 221:1285–1288
3. Gottesman MM, Pastan I (1988) The multidrug transporter, a double edged sword. J Biol Chem 263:12163–12166
4. Gordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR (1990) Expression of the multiple drug resistance gene product (P glycoprotein) in human normal and tumor tissues. Histo Chem Cytochem 38:1277–1283
5. Drach D, Zhao S, Drach J, Mahadevia R, Gattringer C, Huber H, Andreeff M (1992) Subpopulations of normal peripheral blood and bone marrow cells express a functional multidrug resistant phenotype. Blood 11:2729–2735
6. Glennas A, Rugstad HR (1985) Acquired resistance to auranofin in cultured human cells. Scand J Rheumatol 14:230–238
7. Rodenhuis S, Kremer JM, Bertino JR (1987) Increase of dihydrofolate reductase in peripheral blood lymphocytes of RA treated with low dose oral methotrexate. Arthritis Rheum 30: c369–374
8. Norris MD, Haber M, King M, Davey R (1989) Atypical multidrug resistance in CCRF-CEM cells selected for high level methotrexate resistance: reactivity to monoclonal antibody C 219 in the absence of P-glycoprotein expression. Biochem Biophys Res Commun 165:1435–1441
9. Arnett FC, Edworthy SM, Bloch DA (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
10. Ritchie PM, Boyle JA, McInnes JM (1969) Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 11:393–406
11. Chomoczynski P, Sacchin N (1987) Single step method of RNA isolation by acid guandinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
12. Zhang YH, McCabe ER (1992) RNA analysis from newborn screening dried blood specimens. Hum Genet 89:311–34
13. Fuqua SA, Fitzgerald SD, McGuire WL (1990) A simple polymerase chain reaction method for detection and cloning of low abundance transcription. Biotechniques 9:206–211
14. Goldstein LJ, Galski H, Fojo A, Willingham M, Kai SL, Pastan I (1989) Expression of a multiple resistance gene in human cancers. J Natl Cancer Inst 81:116–124
15. Noonan KE, Beck C, Holzmayer TA, Chin JE, Wunder JS, Andrulis JL, Roninson IB (1990) Quantitative analysis of MDR 1 gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci 87:7160–7164
16. Grogan TM, Spier CM, Salmon SE (1993) P-glycoprotein expression in human plasma cell myeloma: correlation with prior chemotherapy. Blood 81:490–495
17. West IC (1990) What determines the substrate specificity of the multi-drug-resistance pump? Trends Biochem Sci 15:42–46
18. Linsenmeyer ME, Jefferson S, Wolf M, Matthews JP, Board PG, Woodcock DM (1992) Levels of expression of the MDR 1 gene and glutathion S transferase genes 2 and 3 and response to chemotherapy in multiple myeloma. Br J Cancer 65:471–475
19. Assaraf YG, Slotky JI (1993) Characterization of a lipophilic antifolate resistance provoked by treatment of mammalian cells with the antiparasitic agent pyrimethamine. J Biol Chem 268:4556–4566
20. Sonnefeld P, Durie BG, Lockhorst HK, Marie JP (1992) Modulation of multidrug resistance multiple myeoloma by cyclosporin. Lancet 340:255–259
21. Tugwell P, Pincus T, Yocum D, Wells G, McKendry R, Kraag G, Baker P (1994) A multi-center double blind randomized trial of low dose cyclosporin and placebo therapy in combination with methotrexate in patients with severe rheumatoid arthritis (abstract). Arthritis Rheum 37:S 391