

Human Endogenous Retrovirus HERV-K(HML-2) Rec Expression and Transcriptional Activities in Normal and Rheumatoid Arthritis Synovia

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ABSTRACT. Objective. Despite abundance in the genome, the possible functions of human endogenous retrovirus (HERV) sequences are not well understood. The involvement of HERV in various disease conditions, such as germ cell tumors or autoimmune diseases like rheumatoid arthritis (RA), has been suggested. We investigated expression of HERV-K(HML-2) *env*-derived transcripts in normal and RA synovia.

Methods. We analyzed HERV-K(HML-2) expression on the mRNA and protein level by RT-PCR analysis and immunofluorescence labeling of the HERV-K(HML-2) Rec (formerly cORF) protein. We examined synovial cell cultures from normal synovia (n = 9), from patients with RA (n = 26), and osteoarthritis (OA, n = 4), and uncultured synovial tissues (RA, n = 12; normal synovia, n = 1).

Results. HERV-K Rec protein was expressed in all normal synovial specimens, and in the majority of RA and OA cases. We demonstrate for the first time expression of HERV-K protein in synovial tissue. RT-PCR and sequence analysis of cloned RT-PCR products confirmed expression of spliced HERV-K(HML-2) *env* transcripts in normal and in arthritic synovia. In addition to *rec* mRNA, several alternatively spliced transcripts, including *np9*, were identified. However, different amounts of the various RT-PCR products indicate different expression levels of HERV-K(HML-2) *env*-derived transcripts in RA compared to normal synovia, with apparently lower expression levels in arthritic synovia.

Conclusion. These findings imply a physiological role of HERV-K(HML-2) Rec in synovial tissue. Differences in the expression of HERV-K *env*-derived transcripts in RA synovia may be caused by disease-specific changes in the general expression pattern. (J Rheumatol 2006;33:16-23)

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HERV-K(HML-2)

Rheumatoid arthritis (RA) is one of the most common classical autoimmune diseases¹. Genetic predisposition for development of RA shown by twin studies and detection of HLA risk alleles is well established¹. The contribution of a wide variety of different factors such as infectious agents or hormonal influences has been suggested or proven in RA¹⁻⁴. The basic pathological mechanisms that ultimately start the characteristic immunologic process, however, remain unresolved.

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A potential role of human endogenous retroviruses (HERV) has recently been implicated in development of RA, based on elevated antibody titers observed in patients with RA⁵. This hypothesis was reinforced by recent data showing an elevated multi-epitope-specific antibody response toward HERV-K proteins in patients with RA⁶, which indicates a protein exposure.

HERV sequences make up about 8% of the human genomic DNA⁷. Evolutionarily they are considered to have entered the genome millions of years ago by germline infections of former exogenous retroviruses⁸. Most HERV are defective because they have accumulated numerous non-sense mutations over time⁸. However, a few exceptions are known. In particular, several proviruses from the biologically most active HERV-K(HML-2) family still display intact retroviral genes for Gag, Prt, Pol, or Env⁸⁻¹⁰. Type 2 proviruses, distinguished by the presence of a 292 bp sequence within the *pol-env* boundary, additionally encode the Rec protein, formerly known as cORF¹¹. Recently the so-called Np9 protein, derived from type 1 proviruses, was identified¹². Both the latter proteins are translated from

splicing products of the proviral mRNA encoded within the *env* gene sequence.

Rec, a functional homolog of the HIV-Rev protein, exports unspliced HERV-K(HML-2) RNA from the nucleus^{11,13}, interacts with the Crm 1 nuclear export factor, and displays functional nuclear export and import signals^{12,14}. Further, Rec protein interacts with the promyelocytic leukemia zinc finger (PLZF) protein, which is involved in mouse spermatogenesis and maintenance of spermatogonial stem cells^{14,15}. There is evidence that Rec may play a role in germ cell tumorigenesis^{12,14}. Np9 protein was found to be expressed in various tumor tissues and transformed cell lines, but not in normal, nontransformed cells¹². The functional conservation of these proteins implies that they provide some physiological function; yet to date the biological significance of intact HERV genes is still mainly unclear. Expression of HERV sequences, on the other hand, has been suspected to play a role in specific disease conditions^{5,16-21}.

Retrovirus-like particles encoded by HERV-K(HML-2) were first identified in teratocarcinoma cell lines²². Expression of HERV-K sequences was subsequently described in various tumors, for example, germ cell tumors⁸, urothelioma, mammary carcinoma, melanoma, and leukemia^{17,23-26}. Patients with germ cell tumors typically display high antibody titers against HERV-K(HML-2) Gag and Env protein²⁷. Elevated antibodies to HERV proteins have as well been reported in various autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, and RA^{5,20,28-30}.

In RA, primarily the synovial compartment is involved in the disease process, as it represents the target as well as the aggressive starting point of the rheumatic changes^{31,32}. We have described a mosaic trisomy 7 as a characteristic cytogenetic finding in RA synovial cells, as well as in OA and to lesser extent in normal synovia³³. Interestingly, a nearly intact member of the HERV-K(HML-2) family was identified on chromosome 7p22³⁴.

With the possible involvement of HERV sequences in autoimmunologic processes in mind, we examined the potential role of HERV-K(HML-2) expression in RA synovia. As the Rec protein is likely to interact with cellular processes because of the functions noted above, we analyzed expression of *rec* mRNA and protein by reverse transcription-polymerase chain reaction (RT-PCR) and immunofluorescence, respectively. Further, we analyzed the expression of other splicing products from the HERV-K(HML-2) *env* gene. We examined HERV-K(HML-2) expression in primary synovial cell lines and uncultured synovial tissue in RA, OA, and normal synovia.

MATERIAL AND METHODS

Synovial specimens were obtained from patients of the Division of Orthopedic Surgery, Bundesknappschaft's Hospital, Puettingen, and from the Department of Orthopedic Surgery, Saarland University, in Homburg/Saar, Germany. Rheumatic and osteoarthritic synovial tissue was

surgically removed from patients with RA or OA for therapeutic reasons. Normal synovia without clinical signs of arthritis or joint disease was obtained by arthroscopy in traumatic joint lesions, from limb amputation, and from autopsy material. Informed consent for research use of tissue material was obtained from all patients. Clinical diagnoses of RA were based on the revised American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria³⁵.

Our study comprised primary synovial cultures of 26 RA, 4 OA, and 9 normal synovial specimens. Synovial tissue was obtained for RT-PCR from one of the RA cases and one uncultured normal specimen. Uncultured tissues from an additional 11 RA cases were also subjected to RT-PCR analysis. The teratocarcinoma cell line Tera 1, which expresses HERV-K(HML-2) sequences at high levels²⁷, served as positive control for analysis of RNA and protein expression.

RT-PCR analysis and immunofluorescence labeling of cultivated cells were performed on parallel cultures of synovial cells, each using identical culture passages. The majority of synovial cultures were analyzed between passages 4 and 12.

RT-PCR was performed for all 39 primary synovial cultures (26 RA, 4 OA, 9 normal) and for 13 uncultured synovial tissues (12 RA, 1 normal). Rec expression on the protein level was examined in 27 cultured synovia specimens (17 RA, 3 OA, 7 normal).

Primary cell cultures. Primary synovial tissue was mechanically dissected, and cells were disaggregated by treatment with 100 U/ml collagenase (Invitrogen, Karlsruhe, Germany) for 4–16 h. Disaggregated cells were seeded in culture flasks, and grown under normal culture conditions in Dulbecco's modified Eagle medium (DMEM) with high glucose (Invitrogen) supplemented with 10% fetal calf serum (Seromed), 1× nonessential amino acids (Invitrogen; 100×), and 1× penicillin/streptomycin (Invitrogen) in a 5% CO₂ atmosphere.

Immunofluorescence. For HERV-K Rec protein-specific immunolabeling a polyclonal Rec-specific rabbit antibody (kindly provided by R. Löwer, Paul-Ehrlich-Institut, Langen, Germany) was used. Cultured synovial cells were grown on glass slides with the above described culture conditions; 48 h before start of immunostaining cells were treated with 3 mM butyrate. After removal of culture media, cells were fixed for 10 min in methanol at –20°C. After permeabilization in phosphate buffered saline (PBS)/0.5% Triton X-100, blocking with 2% bovine serum albumin (BSA) in PBS (20 min at room temperature), and subsequent preincubation with rabbit pre-immune serum (1:500 in Dako antibody diluent; 10–15 min at room temperature) (Dako, Hamburg, Germany), cells were incubated with specific Rec primary antibody diluted 1:100 in PBS/1% BSA for 1 h at room temperature. Primary antibody was detected finally by a fluorochrome conjugated anti-rabbit secondary antibody (goat anti-rabbit/rhodamin; Dianova, Hamburg, Germany). Cellular nuclei were counterstained with DAPI; 200 cells each were analyzed using a Zeiss fluorescence microscope. Digital images were recorded with Metasystems Isis software. Negative control experiments in cultured synovial cells omitting either the Rec primary antibody (but applying BSA blocking and pre-immune serum) or the secondary antibody revealed negative staining results. Evaluation of immunofluorescence labeling was performed comparing test specimens with negative controls and positive controls. Specific staining in test specimens was defined as positive labeling being absent in negative control specimens, which were incubated with rabbit pre-immune serum only (1 h at room temperature after 2% BSA blocking) instead of applying the Rec-immunized rabbit serum, provided the previous described condition, structure-bound staining of nucleoli, as reported¹¹ and observed as characteristic localization of Rec in Rec-positive teratocarcinoma cells, was considered as additional indication of a specific staining.

RNA preparation and RT-PCR. Total RNA was extracted from cultured cells or mechanically dissected freshly frozen tissue using RNA Clean (Hybaid-AGS, Heidelberg, Germany) following the manufacturer's recommendations. RNA was treated with DNase I for 1 h at 37°C. Then 2 µg of RNA was reverse-transcribed in the presence of 25 pmol of random primers

using Omniscript™ (Qiagen, Hilden, Germany). For subsequent PCR amplification HERV-K(HML-2) *env* gene-specific primer sequences were as follows: 5'-ATG AAC CCA TCG GAG ATG CAA-3' and 5'-ACA GAA TCT CAA GGC AGA AG-3'. These primers are identical to the published *env* sequence in the HERV-K(HML-2.HOM) provirus (GenBank accession number AF074086)³⁴. The primers are expected to amplify both full-length as well as spliced *env* transcripts, including the *rec* mRNA. PCR was performed using HotStar Taq Polymerase (Qiagen) with 25 pM primer concentrations. PCR cycling conditions were as follows: 15 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C, with final elongation for 10 min at 72°C. For each sample a 192 bp fragment specific for GAPDH cDNA was amplified as control under the same cycling conditions and using the following primers: 5'-AGT CCA GTG AGC TTC CCG TTC AGC A-3' and 5'-TGG TAT CGT GGA AGG ACT CAT GAC-3'.

Sequence analysis. RT-PCR products of cultured cells from 6 RA and 5 normal synovial samples and the Tera 1 control were subjected to sequence analysis. RT-PCR products were cloned into the pCR4-Topo vector (Invitrogen). Positive clones were purified with Qiagen Miniprep spin columns. A total of 111 RA, 124 normal synovia, and 36 Tera 1 clones were analyzed. Sequencing was done using the Big Dye Terminator Cycle sequencing kit and an ABI 310 Capillary Sequencer (Applied Biosystems, Weiterstadt, Germany). Sequences were evaluated and corrected using the Sequencher program.

RESULTS

Detection of Rec protein by immunofluorescence. Rec-specific immunofluorescence was used to study Rec protein expression in cultured cells from 27 synovial specimens (Table 1). We found that all normal synovial cell cultures (7/7 cases) expressed Rec protein. For individual specimens, Rec labeling was observed in 7.5%–100% of cells, with an average of 52.4%. For RA cases, 94.1% (16/17 cases) of cultured synovial specimens were positive for Rec protein. For individual positive specimens, specific immunolabeling was detected in 5.8%–100% of cells (mean 47.5%). Two of the 3 OA synovial cultures expressed Rec protein, showing 21.1% and 68.8% Rec-positive cells. For all positive synovial specimens, as well as for the Tera 1 positive control, Rec protein was observed in the nucleus, predominantly with nucleolar localization, and to a lesser extent in the cytoplasm (Figure 1). Rarely in individual cells, Rec protein was primarily located in the cytoplasm. A mainly nucleolar localization of Rec protein has previously been described in teratocarcinoma cells¹¹.

RT-PCR from synovial cell cultures. We analyzed HERV-K(HML-2) expression using primers specific for the *env* gene region, including splicing products derived from the *env* gene, such as *rec* and *np9* mRNA. By RT-PCR analysis,

Table 1. Results of Rec immunofluorescence in *in situ* cultivated synovial specimens (17 RA, 3 OA, 7 normal).

	RA	OA	Normal
No. of positive cases	16/17	2/3	7/7
Mean percentage of Rec-positive cells, %	47.5	44.9	52.4
Range of Rec-positive cells, %	5.8–100	21.1–68.8	7.5–100
SD	25.9	33.7	34.7

rec and *np9* mRNA transcripts and other splicing products were observed in all cell cultures derived from normal synovial tissue (9/9), in all OA synovial cultures (4/4), and in all RA synovial specimens but one (25/26) (Figure 2). On a semiquantitative level, RT-PCR product yields from normal synovial specimens were generally higher than from RA and OA specimens, indicating higher expression of HERV-K(HML-2) mRNA in normal synovia compared to RA and OA. In addition to the *rec* mRNA-specific RT-PCR product of 472 bp, 3 different shorter RT-PCR products were observed: 414 bp, about 340 bp, and the recently published *np9* mRNA (254 bp), which is derived from a HERV-K(HML-2) type 1 provirus¹². Full-length *env* transcript, however, was inconsistently detected. RT-PCR conditions were very likely not optimal to amplify an *env* product of about 2.2 kb.

Sequencing of cloned RT-PCR products confirmed the HERV-K(HML-2) specificity, as well as the *rec* and *np9* identity of respective transcripts.

Additional RT-PCR splicing products of 414 bp and about 340 bp (344, 339, 322 bp) that were amplified in a number of samples represent alternative splicing products from the *env* gene that we previously characterized³⁶. Splice donor signals within the *env* gene, located upstream from the *rec*-specific donor signal, are used to produce these alternative splicing products (Figure 3). RT-PCR analysis on the Tera 1-positive control cell line yielded products of 472 bp, 414 bp, and 254 bp.

All 9 normal synovial specimens revealed a similar HERV-K(HML-2) expression pattern, showing the *rec* transcript (472 bp) along with the 254 bp *np9* and the 340 bp cDNA product. The additional 414 bp product was identified inconsistently in 6/9 normal synovial cases (Table 2).

We note that on a semiquantitative level, the various HERV-K(HML-2) mRNA appeared differently expressed in RA and OA cases compared to normal synovia. Spliced HERV-K *env* transcripts were identified in all cultured RA and OA samples, except one RA specimen. HERV-K expression, however, was not as homogeneous as in normal synovial samples. *np9* was amplified the most consistently from all positive RA (25/26, 96.1%) and OA (4/4) cases. In 2 RA cases only *np9* was detected. *rec* mRNA was detected in 23/26 (88.5%) RA and 4/4 OA specimens. The 414 bp product was amplified in 9/26 (34.6%) RA and 1/4 OA cases. Product of about 340 bp, although generally faint, was similarly detected in 9/26 (34.6%) RA cases but no OA (Table 2).

RT-PCR from uncultured synovial tissue. RT-PCR analysis on 13 uncultured synovial tissue samples from 12 RA patients and one normal synovial tissue revealed HERV-K(HML-2) *env*-derived transcripts in 10 (76.9%) specimens. As for cultured synovial cells *rec* and *np9* RT-PCR products, as well as the about 340 bp and 414 bp transcripts were again amplified in variable combinations (Table 3). Apparently HERV-K(HML-2) is expressed independently of

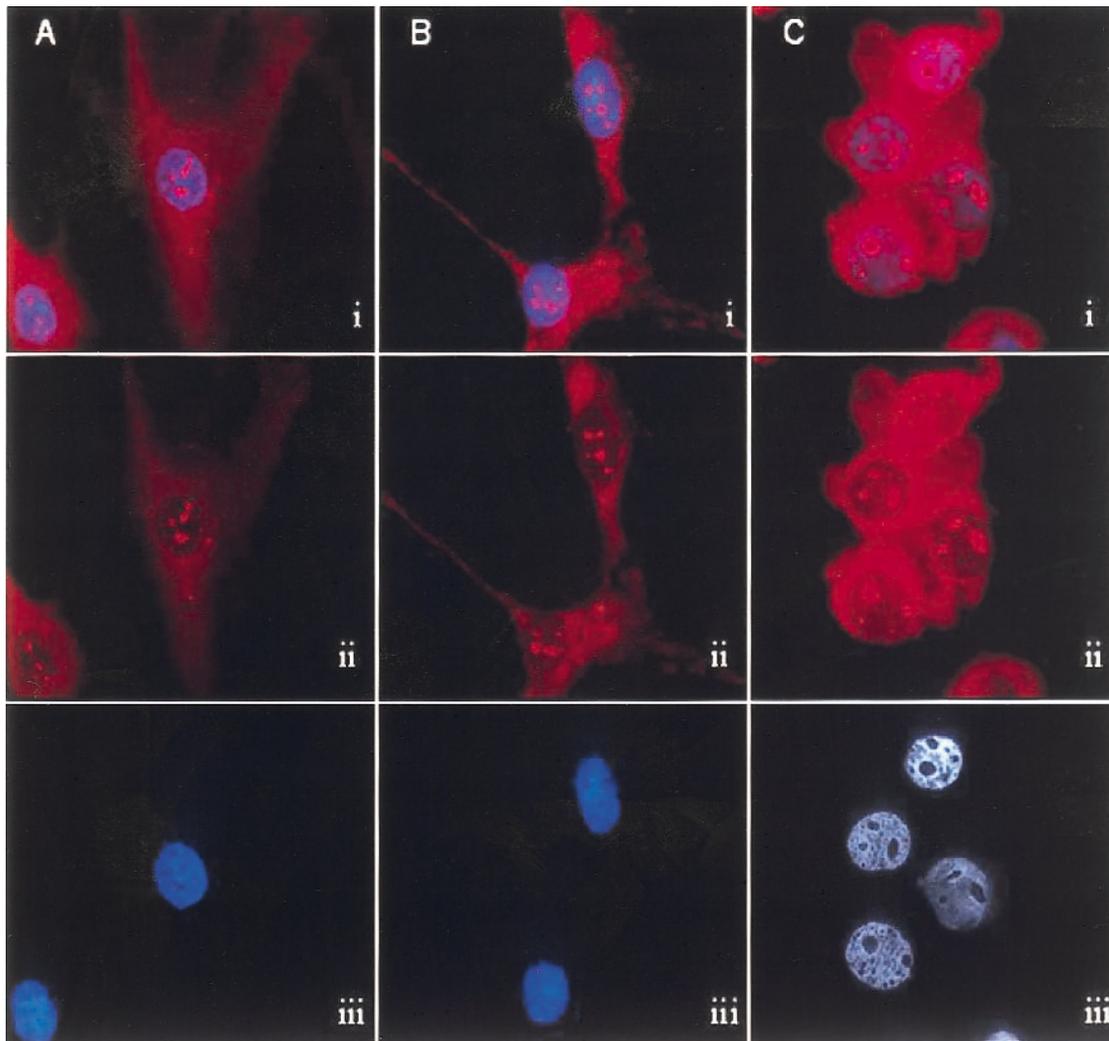


Figure 1. Rec immunofluorescence in cultivated synovial cells with (i) or without (ii) DNA counterstaining (DAPI); DAPI counterstaining (iii). (A) normal synovia (case 31), (B) RA synovia (case 26), (C) Tera 1-positive control cell line. Rec protein is detected predominantly in nucleoli and cytoplasm.

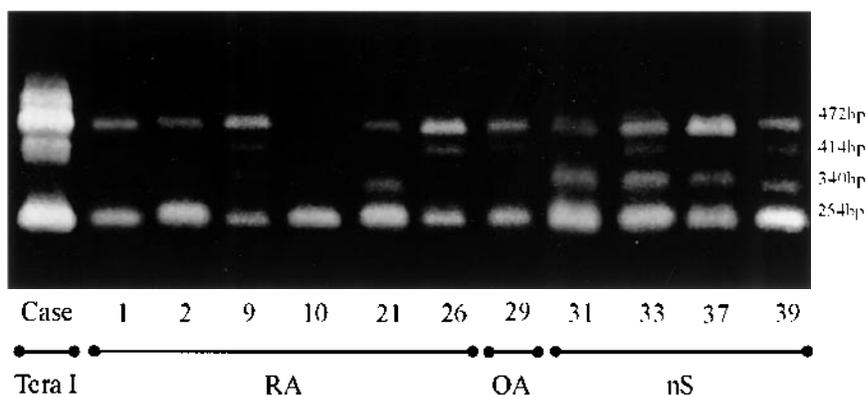


Figure 2. RT-PCR analysis of HERV-K(HML-2) *env*-derived transcripts in cultivated cells from RA, OA, and normal synovia, and from the Tera 1 control cell line expressing *rec* (472 bp), *np9* (254 bp), and other transcripts of about 340 bp and 414 bp. Normal synovial cells show a relatively consistent expression pattern with *rec*, *np9*, and 340 bp transcripts, whereas lower expression levels and a more variable expression pattern are observed in RA and OA synovial specimens. Strong expression of *rec*, *np9*, and 414 bp transcripts is visible in Tera 1 cells.

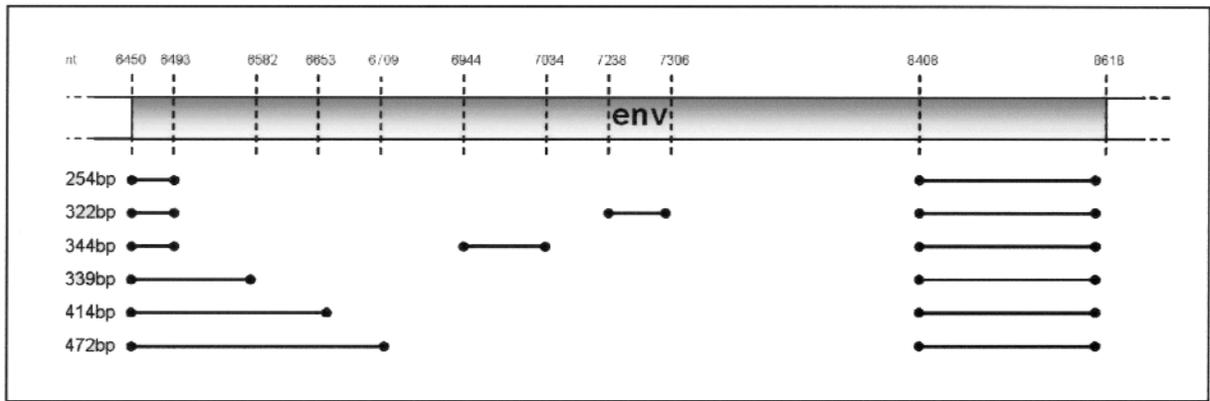


Figure 3. HERV-K(HML-2) *env* transcripts characterized by sequence analysis of RT-PCR products from cultivated RA and normal synovial cells. Respective transcript size and proviral localization of splice acceptor and donor sites are indicated.

cell culture conditions. Moreover, we analyzed HERV-K(HML-2) expression from both cultured and uncultured cells in 2 cases, one RA and one normal specimen (cases 9 and 31, respectively; Table 2). In direct comparison, the 2 uncultured tissue specimens each displayed an almost identical expression pattern of the various HERV-K(HML-2) transcripts compared to the expression pattern after cell culture. Thus, short-term culture of synovial cells apparently did not significantly influence the expression of the HERV-K(HML-2) mRNA we investigated.

As previously reported, HERV-K(HML-2) transcripts could be assigned to a limited number of HERV-K(HML-2) proviruses in the human genome, specifically HERV-K(HML-2) proviruses on chromosomes 7, 12, 6, and 11³⁶.

DISCUSSION

HERV sequences have been repeatedly implicated in autoimmune diseases, such as multiple sclerosis and RA^{5,20}. We describe for the first time expression of HERV-K(HML-2) *env*-derived transcripts in normal and RA synovial cells. Our analysis revealed *rec*, *np9*, and other splicing products from the HERV-K(HML-2) *env* gene were expressed in synovial cells. Similar results from both cultured and uncultured synovial specimens emphasize that HERV-K(HML-2) is expressed *in vivo* in synovial tissue independently of cell culture conditions.

Using a Rec-specific antibody we observed expression of Rec protein specifically in normal as well as rheumatoid synovial cells. Thus our study demonstrates that expression of HERV-K(HML-2) proteins is not restricted to tumor cells.

Further, in synovia, in contrast to previous suggestions⁵, expression of HERV-K(HML-2) genes does not seem to be limited to rheumatoid tissue. Instead, it rather appears to be a physiologic finding. However, the biological function of *rec* (or *np9*) expression in normal and rheumatoid synovial cells remains unclear. In this regard it is interesting, though, that only a limited number of HERV-K(HML-2) proviruses seem to be involved in *rec* expression³⁶.

Only a few HERV loci in the human genome have been found to encode functional proteins⁸⁻¹⁰. An Env protein denoted syncytin³⁷⁻³⁹ from a provirus belonging to the HERV-W family was recently reported to be involved in the development of human placenta, mediating cytotrophoblast fusion. The HERV-K(HML-2) family encodes several conserved retroviral proteins including Np9 and Rec. Andersson, *et al* described expression of HERV-K(HML-2) Rec protein in normal human placenta and at discrete levels in embryonic tissues⁴⁰. Based on EST database analysis, could previously identify HERV-K(HML-2) *rec* transcripts in a melanocytic melanoma cell line, in embryonic stem cells, and in normal retina³⁶.

Our RT-PCR analysis, although only a semiquantitative assessment, revealed different levels of HERV-K(HML-2) *env* gene-derived mRNA in the various specimens. Normal synovia surprisingly displayed a seemingly more constant and stronger gene expression. RT-PCR products of about 340 bp were amplified much less frequently and/or at lower levels from RA and OA cases than from normal synovia. Further differences in the amounts of specific RT-PCR products were observed between normal and rheumatic cells. These RT-PCR results visualized by gel electrophoresis may indicate differential expression of HERV-K(HML-2) *env*-derived transcripts in RA compared to normal synovia in a possibly disease-specific manner. In this context, Nakagawa, *et al*⁴¹ noted differential expression of HERV transcripts in RA compared to normal synovia. Thus, one may speculate that different expression levels of particular HERV-K(HML-2) transcripts play a role in RA. On the other hand, secondary nonspecific expression changes due to general alteration of the cellular metabolism and expression pattern in arthritic synovia might be possible.

A pathogenic influence of HERV expression in RA has been suggested from serologic data. Patients with RA were reported to display an impaired immune response toward a variety of antigens and autoantigens⁴². An increased antibody response toward HERV-K proteins reported in patients

Table 2. RT-PCR analysis of spliced HERV-K(HML-2) env transcripts in 39 synovial cell cultures (26 RA, 4 OA, 9 normal).

Case	Age, yrs Sex	Clinical Diagnosis	472 bp	414 bp	340 bp	254 bp
1	81 M	RA	+			+
2	40 F	RA	+			+
3	72 F	RA				
4	64 M	RA	+			+
5	75 F	RA	+			+
6	54 F	RA	+	(+)	+	+
7	66 F	RA	+	(+)		+
8	51 F	RA	+			+
9*	39 F	RA	+	(+)	(+)	+
10	73 F	RA				+
11	69 F	RA	+			+
12	43 M	RA	+	(+)		+
13	47 F	RA	+		(+)	+
14	40 F	RA	+	(+)		+
15	65 F	RA	+		+	+
16	35 F	RA	+		(+)	+
17	58 F	RA	+		(+)	+
18	67 F	RA	+	(+)		+
19	52 F	RA	+	(+)		+
20	62 F	RA	+	(+)		+
21	41 F	RA	+		+	+
22	68 F	RA	+		+	+
23	46 F	RA	+		+	+
24	66 F	RA	(+)			+
25	40 F	RA				+
26	32 F	RA	+	+		+
27	71 M	OA	+	+		+
28	63 F	OA	+	(+)		+
29	66 F	OA	+			+
30	68 F	OA	+			+
31*	17 M	Normal	+		+	+
32	54 M	Normal	+		+	+
33	53 F	Normal	+	(+)	+	+
34	28 F	Normal	+	(+)	+	+
35	74 F	Normal	+	(+)	+	+
36	47 F	Normal	+	+	(+)	+
37	61 M	Normal	+		+	+
38	59 M	Normal	+	(+)	+	+
39	47 M	Normal	+	(+)	+	+
Control	-/M	Tera1	+	+		+

* RT-PCR analysis of uncultivated synovia was performed from these specimens as well (see Table 3). Tera 1: teratocarcinoma cell line, +: detection of transcript by gel electrophoresis, (+): weak detection of transcript by gel electrophoresis.

with autoimmune arthritis^{5,6} could be interpreted as autoimmunologic failure. A multi-epitope response toward HERV-K(HML-2) proteins, as reported for RA patients, makes a protein exposure likely⁶. In our study HERV-K expression and specifically Rec protein was present in both normal and rheumatoid synovial tissue. This is compatible with the observation of HERV-K(HML-2)-specific antibodies appearing also in healthy individuals⁶. In chronic arthritic disease, inflammatory processes cause increased blood circulation and cell and tissue destruction in the affected joint⁴³. By this process, synovial HERV-K(HML-2) proteins that are usually hidden might be increasingly presented to

immune cells and thus might trigger an elevated immune response in patients with RA.

Although the expression of HERV-K protein in itself does not seem to be a primary pathogenic factor in RA, an altered T or B cell response to respective proteins expressed in RA synovial tissue might focus the autoimmunologic inflammation process on the joint. Thus HERV-K proteins, like other autoantigens⁴², may yet play a role in secondary pathologic mechanisms in RA. An immune response with elevated serum antibodies has been described for several HERV genes^{5,6}. Serum analyses targeting HERV-K Rec antibodies and investigation of a respective T or B cell

Table 3. RT-PCR analysis of spliced HERV-K(HML-2) *env* transcripts from 13 uncultivated synovial tissues (12 RA, 1 normal).

Case	Clinical Diagnosis	472 bp	414 bp	340 bp	254 bp
9*	RA-T	+		+	+
40	RA-T	+			+
41	RA-T	+	+	+	+
42	RA-T	+			+
43	RA-T			+	+
44	RA-T				
45	RA-T	+			+
46	RA-T				
47	RA-T			+	+
48	RA-T	+	(+)		+
49	RA-T				
50	RA-T			+	+
31*	Normal-T	+		+	+

* RT-PCR analysis of cultivated synovia was performed for these specimens as well (see Table 2). Due to anonymized samples, age and sex could not be assigned to the individual cases 41–50. T: uncultivated synovial tissue, +: detection of transcript by gel electrophoresis, (+): weak detection of transcript by gel electrophoresis.

response in synovial tissues in patients with RA are needed to elucidate these issues.

HERV-K(HML-2) *env* gene-derived mRNA and the Rec protein are expressed in normal and in rheumatoid synovial cells, indicating a physiological function of at least one conserved HERV-K protein in synovial tissue. A pathogenic effect of HERV-K expression suggested as a potential initiating factor in autoimmune disease could not be proven in RA. On the contrary, expression of specific HERV-K sequences seems to be a healthy condition in synovial tissue. Transcription levels of particular transcripts differ between normal and RA synovial specimens. Reduction or changes of HERV-K(HML-2) expression in the arthritic synovia might occur due to more general changes in the overall cellular expression pattern. Disease-specific different transcriptional regulation of HERV-K(HML-2) in rheumatoid compared to normal synovial tissue, however, may still be considered and merits further investigation.

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