Identification of Self-Epitopes Recognized by T Cells in Rheumatoid Arthritis Demonstrates Matrix Metalloproteinases as a Novel T Cell Target

JESSICA ter STEEGE, MARIEKE VIANEN, JOLANDA van BILSEN, JOHANNES BIJLSMA, FLORIS LAFEBER, and MARCA WAUBEN

ABSTRACT. ***Objective.*** To identify novel arthritis-associated and/or cartilage-specific self-epitopes recognized by T cells in patients with rheumatoid arthritis (RA).

Methods. Human analogs of several self-epitopes recognized in the rat adjuvant arthritis (AA) model (n = 13) were tested for T cell recognition in patients with RA and healthy controls. Recognition was assessed by proliferative activity of peripheral blood mononuclear cells (PBMC). In addition, cytokine production was determined.

Results. Six out of the 13 peptides recognized during AA were also recognized by more than 20% of the RA patients, in contrast to only one out of the 16 control peptides that were not recognized during AA. The highest proliferative responses were to matrix metalloproteinase (MMP)-derived peptides. The response to a MMP-1 epitope was significantly higher in RA patients than in healthy controls. Moreover, this MMP-1 epitope increased interleukin 4 (IL-4) production of RA PBMC and decreased IL-4 production by control PBMC. The proliferative response to a MMP-3 epitope was similar in RA patients and controls; however, the MMP-3 epitope increased IL-4, and concomitantly IL-1ß and tumor necrosis factor-α production of RA PBMC, whereas these cytokines were unaffected in control PBMC.

Conclusion. This study shows the presence of immune reactions to MMP-derived T cell epitopes that are associated with RA, suggesting a novel role of MMP in RA. (J Rheumatol 2003;30:1147–56)

Key Indexing Terms:
RHEUMATOID ARTHRITIS MATRIX METALLOPROTEINASE MMP-1 AUTOANTIGEN

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease leading to joint destruction. Inflammation in the joint is characterized by infiltration of CD4+ T cells and activation of synoviocytes, macrophages, and B cells[1]. The presence of large numbers of activated T cells and MHC class II-expressing cells, in addition to the association of RA with HLA-DR alleles, suggests an ongoing antigen driven response. It has been proposed that the CD4+ T cells play a significant role in the pathogenesis of RA[1-4]. Therefore, identification of antigens driving or controlling the T cell response is of great importance.

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At present a limited number of self-antigens, such as proteoglycan[5,6], collagen type II[7], gp39[8], and synovial fluid-derived p205[9] as well as heat shock proteins[4,10,11] have been implicated in the pathogenesis of RA. It has been hypothesized that autoreactive T cells can be triggered by presentation of viral or bacterial antigens displaying sufficient homology to a self-antigen to cause cross-reactivity, yet differ enough to break tolerance (molecular mimicry hypothesis)[11-14]. Clinical observations suggest that the presence of articular cartilage is a critical factor for the ongoing inflammatory response in RA[15]. Therefore, it is of particular significance to determine whether T cells of patients with RA respond to cartilage-derived components[16,17].

Besides such activation of autoaggressive T cell responses, the lack or dysfunction of regulatory T cells involved in the maintenance of peripheral tolerance could play a pivotal role in the initiation and/or maintenance of the disease process[4]. In general the balance between Th1 and Th2 T cells, as represented by their specific interferon-γ (IFN-γ) and interleukin 4 (IL-4) production, respectively, seems to be part of the pathogenesis of RA[3,18]. A relative predominance of Th1 cell activity mediates macrophage activation, leading to production of IL-1ß and tumor necrosis factor-α (TNF-α), cytokines involved in cartilage damage, as observed in the joints of patients with RA[18-20].

Identification of self-epitopes involved in the pathogen-

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esis of RA and characterization of T cell responses during the disease process will provide important information on the role of T cells in RA. Furthermore, such self-epitopes will enable us to monitor RA-specific T cell responses during the course of the disease, and may be important targets for immunotherapy.

This study was conducted to identify arthritis-related and/or cartilage-specific self-antigens that induce T cell reactivity in RA patients. We investigated T cell responses of RA patients to self-peptides that have been recently identified as T cell epitopes recognized during experimental arthritis in rats[21]. These novel T cell epitopes were selected by a computer search strategy to arthritis and/or cartilage-associated rat proteins based on the molecular mimicry theory in adjuvant arthritis (AA)[21]. In our study, human analogs of these peptides were tested for recognition by peripheral blood mononuclear cells (PBMC) of RA patients and healthy controls. In addition to proliferation-inducing activity of PBMC, the peptides were tested for their ability to influence the production of T cell and macrophage-derived cytokines.

MATERIALS AND METHODS

Peptide selection and synthesis. The human analogs of 29 peptides were selected on the basis of the molecular mimicry computer search described by van Bilsen, *et al*[21]. Briefly, the interaction of the mycobacterial heat shock protein 65 (hsp65) 178–186 peptide with rat MHC class II ($RT1.B^L$) and the T cell receptor of an arthritogenic T cell clone was used as a mold for the identification of fitting self-epitopes[22,23]. Nine sequential amino acids present in arthritis and/or cartilage-associated proteins were selected from a database, and these sequences appeared to be conserved, differing at most in 3 amino acids from the human sequence. Human analogs of these peptides (n = 29) were synthesized. To enhance the likelihood for binding to human MHC class II, the 9-mer-selected peptides were extended to 15-mer peptides, by adding the 3 naturally occurring amino acids at the N and C terminus. The peptides were assembled by automated simultaneous multiple peptide synthesis as described[24]. Briefly, standard Fmoc chemistry with *in situ* PyBop/NMM activation of the amino acids in a 5-fold molar excess with respect to 2 µmol/peptide PAL-PEG-PS resin (Perceptive Biosystems, Maarssen, The Netherlands) was employed. Peptides were obtained as C-terminal amides after cleavage with 90–95% TFA scavenger cocktails. Peptides were analyzed by reversed phase high performance liquid chromatography (HPLC) and checked via electrospray mass spectrometry on an ion-trap mass spectrometer (LCQ; Thermoquest, Breda, The Netherlands). If the peptide purity was less than 75%, peptides were purified on reverse phase HPLC before use. Tables 1 and 2 show the sequences of 2 panels of peptides fulfilling the search criteria as described by van Bilsen, *et al*[21], and the corresponding amino acid positions in the protein. Peptides of panel 1 are the 13 peptides recognized during AA (Table 1), while peptides of panel 2 are 16 randomly selected peptides fulfilling the search criteria, but which were not recognized during AA (Table 2).

Patients and controls. For analysis of T cell responses, PBMC were isolated from 98 randomly selected RA patients visiting the outpatient clinic of University Medical Center Utrecht. RA was defined according to the 1987 revised American College of Rheumatology criteria[25]. Patient characteristics are given in Table 3. As expected, it concerned a heterogeneous group of RA patients; nevertheless we compared various categories of patients (e.g., using similar medication) with regard to the T cell variables measured.

A group of healthy volunteers (laboratory workers and family members) was used as controls. This group consisted of 35 persons similar for sex (28 women, 7 men) and age (30 to 83 yrs, mean 54 ± 12) to the patient group.

Cell isolation and activation. PBMC were isolated by Ficoll density gradient centrifugation of heparinized blood (Pharmacia, Uppsala, Sweden). Viability of the cells, checked by trypan blue exclusion, always exceeded 95%. Cells were cultured in RPMI 1640 supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco BRL, Gaithersburg, MD, USA), and 10% pooled A+ human serum (Red Cross Blood Bank Utrecht, The Netherlands).

To assess proliferative activity, cells were cultured (1×10^5 cells/well, 200 µl) in quadruplicate in flat-bottom 96 well plates (Nunc, Roskilde, Denmark) for 5 days at 37°C and 5% CO_2, in the absence or presence of 1 or 10 µg/ml peptide. During the last 18 h of culture 1 µCi ^{3}H-thymidine (Amersham, Buckinghamshire, UK) was added to each well. Cells were harvested according to standard procedures and incorporated radioactivity was measured by liquid scintillation counting (Wallac 1205 beta plate liquid scintillation counter, Oy, Finland) and expressed as counts per minute (cpm). The proliferative response is expressed as stimulation index (SI), calculated as the mean cpm of cells cultured with peptide divided by the mean cpm of cells cultured without peptide.

For analysis of cytokine production by PBMC, cells were cultured (0.5×10^6 cells/well, 1 ml) in duplicate in 24 well plates (Nunc) for 3 days in the absence or presence of 1 or 10 µg/ml peptide. Spontaneous production of T cell cytokines such as IFN-γ and IL-4 by these PBMC was found to be low or absent. Therefore, after antigen stimulation, T cell cytokine production was nonspecifically enhanced using mitogen stimulation for the last 48 h in one of the duplicate wells [anti-CD3/anti-CD28; CLB-T3/4.E (1.5 µg/ml) and CLB-CD28 (2.0 µg/ml) (CLB, Amsterdam, The Netherlands)] as described[26-28]. This stimulation activates T cells through the CD3 complex together with the major costimulatory CD28-mediated signal. Subsequently, the supernatants were harvested and freed of cellular material by centrifugation (5 min, 900 g), frozen in liquid nitrogen, and stored at –20°C. IL-1ß and TNF-α production were determined in samples without additional stimulation. All cytokines were determined by ELISA according to the manufacturer's instructions (Biosource, Ettenleur, The Netherlands). The detection limits were 40 pg/ml for IFN-γ, 20 pg/ml for TNF-α, and 10 pg/ml for IL-4 and IL-1ß.

Statistical analysis. Statistical evaluation was performed using SPSS software, version 7.5 (SPSS, Chicago, IL, USA). The Mann-Whitney U test, Fischer's exact test, Wilcoxon test, Kruskal-Wallis test, or logistic regression analysis were used for analysis of statistical significance when appropriate. Determination of correlations between the various parameters was by Spearman correlation test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Proliferative responses of PBMC in patients with RA and controls. To analyze T cell reactivity in a broad variety of patients with RA, we selected a random patient group differing in disease duration, severity, rheumatic factor, medication, and putative genetic background (Table 3). The 2 panels of 29 total human self-peptides were screened for induction of proliferation of PBMC of 25 patients. Peptides were tested in a concentration of 1 and 10 µg/ml. Six out of the 13 peptides of Table 1 (of which the rat analogs were recognized during the course of AA) induced a proliferative response (SI ≥ 2) in more than 20% of the patients. In contrast, only one out of the 16 peptides of Table 2 (of which the rat analogs were selected via the search profile but were not recognized during AA) induced a proliferative response

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Table 1. Motif peptides recognized during adjuvant arthritis (Panel 1)*.

Protein	Abbreviation	Position**	Sequence
Aggrecan core protein precursor	Aggrecan (2)	1352–1366	LSGLPSGGEVLEISV
Chondroitin sulfate proteoglycan NG2 precursor	Chondr S1	594–608	QLLGVSASVPVEHRD†
Chondroitin sulfate proteoglycan NG2 precursor	Chondr S2	1862–1876	VVDPDSAPGEIEYEV†
Fibrillin-1 precursor	Fibrillin	1526–1540	CVDTRSGNCYLDIRP
Laminin alpha-1 chain precursor	Laminin (1)	906–920	VKGSHSAVCHLETGL
Laminin alpha-1 chain precursor	Laminin (2)	1458–1472	PPASFSPTCVLEGDH
Lumican precursor	Lumican	266–280	LKNIPTVNENLENYY
Matrix metalloproteinase-1	MMP-1	14–28	GVVSHSFPATLETQE
Matrix metalloproteinase-3 (2)	MMP-3	446–460	FFYFFTGSSQLEFDP
Matrix metalloproteinase-10	MMP-10	329–343	SAFWPSLPSYLDAAY
Matrix metalloproteinase-16	MMP-16	539–553	VKEGHSPPDDVDIVI
Perlecan	Perlecan	3018–3032	SYRLRSPVISIDPPS
Tenascin	Tenascin	1789–1803	EPVSGSFTTALDGPS

* All peptides fulfill the computer search criteria described by van Bilsen, *et al* [21]. ** Amino acid numbering according to the Swiss Protein Database. † Since no human sequences were available for these peptides, rat sequences were tested.

Table 2. Motif peptides that are not recognized during adjuvant arthritis (Panel 2)*.

Protein	Abbreviation	Position**	Sequence
Aggrecan core protein precursor	Aggrecan (1)	1479–1493	EDLVGSASGDLDLGK†
Aggrecan core protein precursor	Aggrecan (3)	924–938	ISGLPSGGDDLETST†
Alpha 2-macroglobulin	Alpha 2	225–238	KEKLRSINQGLDRLR
Bone morphogenetic protein 8 precursor	BMP	102–116	ADLVMSFVNMVERDR
Calpain-2	Calpain	262–276	HAYSVTGAEEVESNG
Chondroitin sulfate proteoglycan NG2 precursor	Chondr S3	346–360	GAANISLVGCIEDFS†
Chondroitin sulfate proteoglycan NG2 precursor	Chondr S4	1146–1160	QGTIDTAVLHLDTNL†
Dekprotein	Dekprotein	154–168	NAMLKSICEVLDLER
Fibromodulin	Fibromodulin	327–341	EFSISSFCTVVDVVN
Fibronectin precursor	Fibronectin	1622–1636	QPLVQTAVTNIDRPK
Laminin alpha-1 chain precursor	Laminin (3)	2179–2193	LWDLGSGSTRLEFPD
Matrix metalloproteinase-3 (1)	MMP-3 (1)	330–344	SSFWPSLPSGVDAAY
Phospholipase A_2	Phosp A2	53–67	LGGSGTPVDELDKCC
Procollagen alpha-1 (III) chain precursor	Collagen-1	1235–1249	MTSLKSVNGQIESLI
Procollagen alpha-2 (V) chain precursor	Collagen-2	1266–1280	HATLKSLSSQIETMR
Versican core protein precursor	Versican	1846–1860	TTTVSSFSLNVEYAI

* All peptides fulfill the computer search criteria described by van Bilsen, *et al* [21]. ** Amino acid numbering according to the Swiss Protein Database. † Since no human sequences were available for these peptides, rat sequences were tested.

Table 3. Patient characteristics. Erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and antiperinuclear factor (APF) were obtained in retrospect and were available for 85, 94, and 49% of patients, respectively.

Age, yrs	60 ± 13 (19–95)*
Male/female	24/74
Disease duration, yrs	14 ± 12 (0.5–50)*
RF positive, %**	56
APF positive, %**	61
ESR, mm/h	31 ± 25 (4–96)*
NSAID use, %**	82
DMARD use, %**	71
MTX use, %**	37
Prednisone use, %**	20
Combination therapy, %**	77

* Mean ± SD (range). ** Expressed as percentage of patients. NSAID: nonsteroidal antiinflammatory drug, DMARD: disease-modifying antirheumatic drug, MTX: methotrexate.

(SI ≥ 2) in more than 20% of the patients. Next, the proliferation-inducing capacity of these 7 peptides was further analyzed in 39 patients, except for the MMP-3 peptide that was tested in 98 patients, using the optimal peptide concentration for each peptide. The final frequency of PBMC stimulation (proliferative response SI ≥ 2) is shown in Figure 1A. The highest frequencies were observed for peptides derived from MMP-1, MMP-3, and MMP-16. Respectively, 32, 40, and 32% of all RA patients tested responded to these MMP peptides. Furthermore, determination of the mean SI of all patients tested indicated that these 3 MMP peptides induced the most pronounced proliferation (Figure 1B). The observed increase in cpm was statistically significant for MMP-1, MMP-3, and MMP-16, with p values ≤ 0.001, ≤ 0.01, and ≤ 0.01, respectively.

To investigate whether the proliferative responses were

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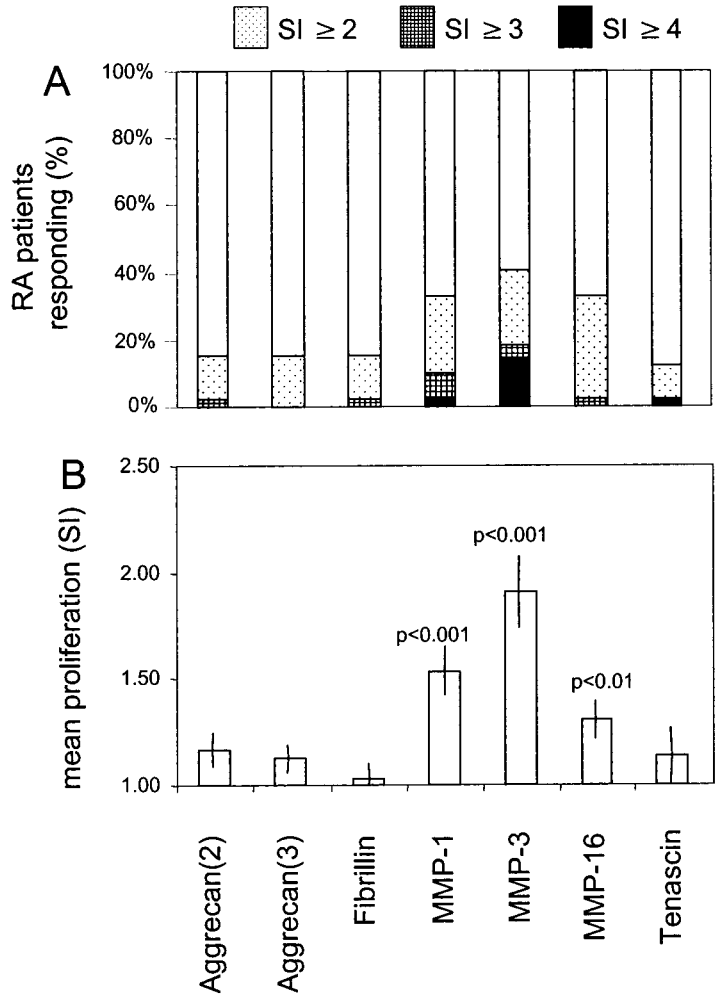

Figure 1. Peptide-induced proliferation of PBMC obtained from patients with RA (n ≥ 39). A. Percentage of patients showing a proliferative response (SI ≥ 2). Highest frequencies were found for peptides from MMP-1, MMP-3, and MMP-16. B. Proliferative response given as the mean SI (± SEM) of all tested patients. Significant proliferation was observed for MMP-1, MMP-3, and MMP-16, as indicated by p values (Wilcoxon test, $p < 0.05$), determined by paired analysis of the mean cpm of the culture without peptide and the mean cpm of culture with peptide. The optimal peptide concentrations shown are 1 µg/ml for aggrecan (2), MMP-1, and tenascin, and 10 µg/ml for aggrecan (3), fibrillin, MMP-3, and MMP-16. Basal cpm value for RA patients was 410 (± 266).

associated with RA patients, the peptides were also tested for proliferation induction of PBMC obtained from 20 healthy controls, except for MMP-3, which was tested in 35 controls. The frequency of PBMC stimulation (proliferative response SI ≥ 2) of controls is shown in Figure 2A. The mean SI of PBMC cultures of all donors is shown in Figure 2B. As in RA patients, the peptides from MMP-3 and MMP-16 were recognized in a high percentage (frequency > 30%) of the controls, in contrast to the MMP-1 peptide. The mean cpm of MMP-3 and MMP-16-induced proliferation was significantly increased compared to control cultures ($p \leq 0.05$).

Importantly, the frequency of the MMP-1 peptide-induced proliferation was higher in RA patients compared to controls (Fischer's exact test, $p \leq 0.06$). Also, the mean proliferative response (SI) to the MMP-1 peptide was significantly higher in RA patients compared to controls (Mann-Whitney U, $p \leq 0.05$). Multiple regression analysis was performed to evaluate the possible contribution of age in the MMP-1-induced difference in proliferation between RA patients and controls; no relation with age was found ($p > 0.349$).

Peptide-induced proliferation related to patients' characteristics and disease measures. Proliferation induced by the peptides from MMP-1 and MMP-16 showed no significant relationship with patients' characteristics or disease vari-

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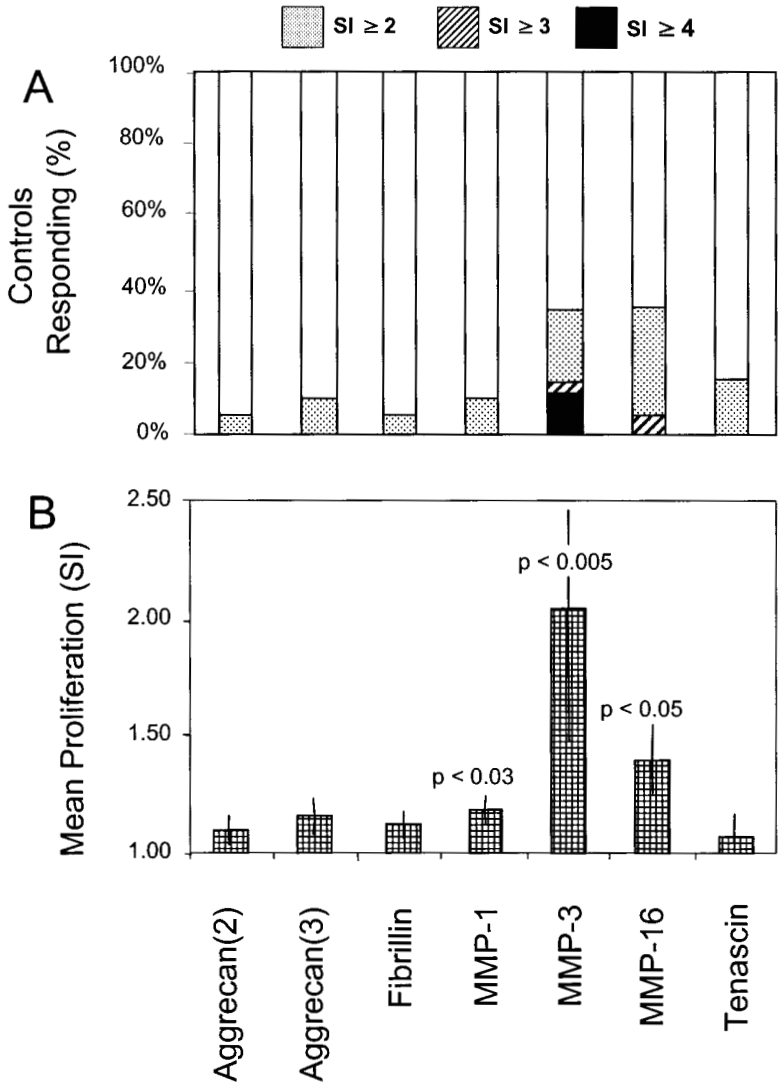

Figure 2. Peptide-induced proliferation of PBMC obtained from healthy controls (n ≥ 20). A. Percentage of controls showing a proliferative response (SI ≥ 2). Highest frequencies were found for the peptides from MMP-3 and MMP-16. B. Proliferative response given as the mean SI (± SEM) of all tested controls. Proliferation was significant for MMP-1, MMP-3, and MMP-16 (Wilcoxon test, $p \leq 0.05$). P values are determined by paired analysis of the mean cpm of the culture without peptide and the mean cpm of culture with peptide. Optimal peptide concentration shown was 1 µg/ml for aggrecan (2), MMP-1, and tenascin, and 10 µg/ml for aggrecan (3), fibrillin, MMP-3, and MMP-16. Basal cpm value for controls was 661 (± 268).

ables. In contrast, MMP-3-induced proliferation correlated negatively with disease duration, age, and erythrocyte sedimentation rate (ESR), and other variables did not correlate (Table 4). Also, when proliferative activity was dichotomized (SI non-responsive < 2 < SI responsive), it appeared that responders had the lowest disease duration, age, and ESR ($p \leq 0.007$, ≤ 0.001, ≤ 0.007, respectively). Multiple regression analysis revealed that disease duration determined the most significantly the difference in MMP-3-induced proliferation ($p \leq 0.006$). Moreover, since there was no correlation between MMP-3-induced proliferation and age in the control group ($r = 0.112$, $p \geq 0.522$) the MMP-3-induced proliferation is not expected to be merely an age-related phenomenon.

Cytokine production induced by MMP peptides. The 3 MMP peptides inducing the strongest proliferative responses were selected to analyze cytokine production of RA and control PBMC upon peptide incubation.

As shown in Figure 3, peptides MMP-1 and MMP-3 induced a significant stimulation of IL-4 production by

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Table 4. Patients' characteristics and disease data correlated with immunological variables. Correlation coefficients and p values of regression analysis are given. When p values were > 0.25, NS is indicated as being not statistically significant. When dichotomizing proliferation in responders and nonresponders using an SI of 2 as a cutoff point, disease duration, age, and ESR were statistically significantly lower in the responder group.

	Disease Duration	Age	ESR
MMP-1			
SI	NS	NS	NS
IL-4	+ 0.683, p < 0.04	NS	NS
IFN	NS	NS	NS
TNF	NS	NS	NS
IL-1	NS	NS	NS
MMP-3			
SI	–0.270, p < 0.07	–0.369, p < 0.001	–0.238, p < 0.03
IL-4	+0.317, p < 0.10	NS	NS
IFN	NS	NS	NS
TNF	NS	NS	NS
IL-1	NS	NS	NS
MMP-16			
SI	NS	NS	NS
IL-4	NS	NS	NS
IFN	NS	NS	NS
TNF	NS	NS	NS
IL-1	NS	NS	NS

SI: stimulation index, IL: interleukin, IFN: interferon, TNF: tumor necrosis factor.

PBMC of patients with RA. Interestingly, this response was observed in all patients after stimulation with MMP-1, whereas 74% of the patients showed an increase in IL-4 production after stimulation with MMP-3. In contrast, in control PBMC, MMP-1 induced a significant inhibition of IL-4 production (observed in 90% of the controls), while MMP-3 stimulation had no effect on IL-4 production in controls. For MMP-1 the difference in IL-4 production between patients and controls was statistically significant and was not an age-related phenomenon, as shown with multiple regression analysis ($p \geq 0.646$). Peptide MMP-16 did not affect the IL-4 production in either patients or controls.

Both MMP-1 ($p < 0.062$) and MMP-3 induced a slight, statistically insignificant inhibition of IFN-γ production in RA patients' PBMC. In controls, IFN-γ production remained unchanged by MMP-3, but was significantly diminished by addition of MMP-1 (Figure 3). MMP-16 had no effect on IFN-γ production in patients or controls.

Addition of MMP-3 peptide increased both IL-1ß ($p < 0.031$) and TNF-α ($p < 0.006$) production of PBMC from RA patients (an elevation in more than 70% of the patients), while no effect was seen in controls' PBMC (Figure 4). MMP-1 peptide had no effect on TNF-α production of RA PBMC, whereas in control PBMC an inhibition of TNF-α production was observed (Figure 4). The peptide of MMP-16 did not affect TNF-α and IL-1ß levels of either RA or control PBMC.

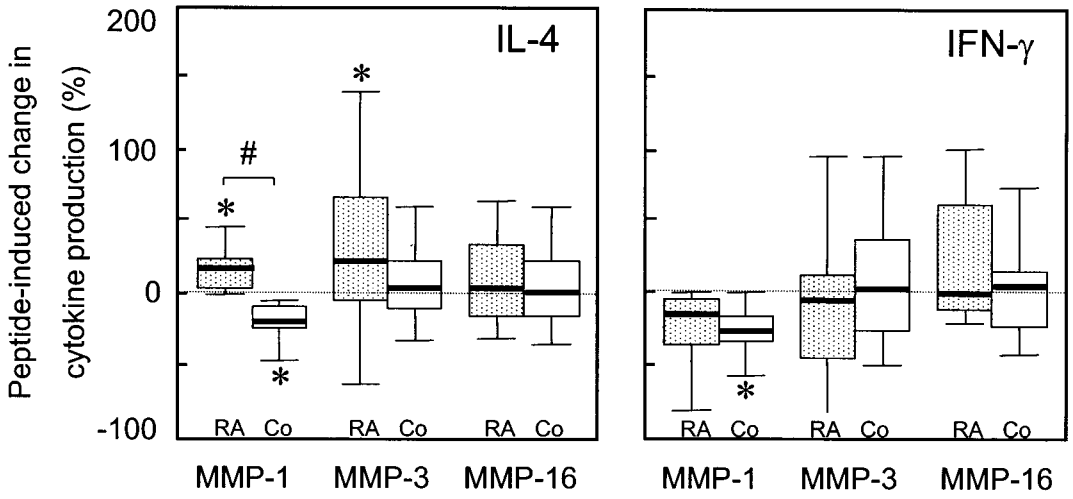

Figure 3. IL-4 and IFN-γ production induced by MMP-1, MMP-3, and MMP-16. Peptide-induced cytokine production is expressed as percentage of cytokine production detected in supernatants of corresponding PBMC cultures without peptide. PBMC were cultured for 72 h. After 24 h the T cell-specific stimulus anti-CD3/anti-CD28 was added to enhance cytokine production. Median values and 25–75th (boxes) and 10–90th (lines) percentiles for RA patients and controls (Co) are shown. MMP-1 and MMP-16-induced cytokine production was tested in 18 RA patients and 20 controls. MMP-3-induced cytokine production was tested in 37 RA patients and 35 controls. *Significant change in cytokine production after peptide stimulation compared to corresponding PBMC culture without peptide (Wilcoxon, $p \leq 0.05$). #Significant difference in effect on cytokine production between RA patients and controls (Kruskal-Wallis, $p \leq 0.05$). Basal levels of IL-4 and IFN-γ after anti-CD3/CD28 stimulation did not statistically differ between patients and controls. Median basal IL-4 production was 110 pg/ml (range 42–511) for patients, 126 pg/ml (range 22–355) in controls. Median basal IFN-γ production was 27 ng/ml (range 1–268) in patients, 22 ng/ml (range 2–38) in controls.

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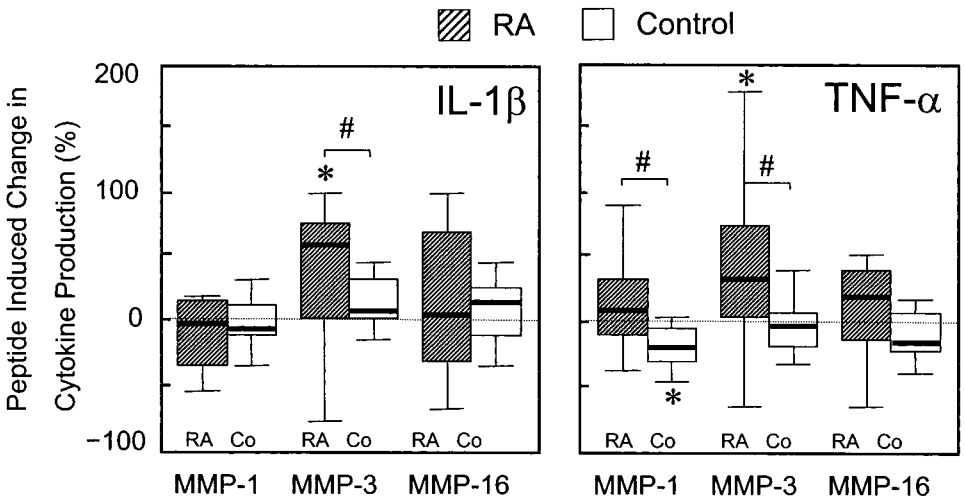

Figure 4. IL-1ß and TNF-α production induced by MMP-1, MMP-3, and MMP-16. Peptide-induced cytokine production is expressed as percentage of cytokine production detected in supernatant of corresponding PBMC cultures without peptide. PBMC were cultured 72 h. Median values and 25–75th (boxes) and 10–90th (lines) percentiles for RA patients and controls (Co) are shown. MMP-1 and MMP-16-induced cytokine production was tested in 18 patients and 20 controls. MMP-3-induced cytokine production was tested in 37 patients and 20 controls. *Significant change in cytokine production after peptide stimulation compared to corresponding PBMC culture without peptide (Wilcoxon, $p \leq 0.05$). #Significant difference in cytokine effect between patients and controls (Kruskal-Wallis, $p \leq 0.05$).

Peptide-induced cytokine production related to disease variables. MMP-1-induced IL-4 production of PBMC from RA patients correlated positively with disease duration ($r = 0.683$, $p \leq 0.04$), and a similar trend was observed for MMP-3-induced IL-4 production ($r = 0.317$, $p \leq 0.1$; Table 4). The MMP-3 and MMP-1-induced IL-1ß and TNF-α production did not correlate significantly with disease variables. For the control group, no associations were found for any of the peptide-induced changes in cytokine production and age.

DISCUSSION

This study was conducted to identify arthritis-related and/or cartilage-specific T cell epitopes that induce T cell reactivity in patients with RA. We investigated T cell responses of RA patients to peptides identified as T cell epitopes in experimental arthritis in rats[21]. The selected rat peptides contained conserved sequences differing in less than 3 amino acids from the human sequences. This observation encouraged us to evaluate T cell recognition of the human analogs in RA.

In vitro analysis of PBMC proliferative responses to putative autoantigens in RA has been shown to be cumbersome. It has been reported that the ability of T cells of RA patients to respond to antigens is downregulated[29,30], or that RA T cells can be partly anergic[31]. Further, the use of synthetic peptides, lacking chemical modifications that may occur in native epitopes, may not be adequate to detect T cell responses[32]. However, several human cartilage gp39[8] and collagen[33] epitopes have been successfully identified, based on synthetic peptide-induced proliferation of RA PBMC. In our study proliferative responses to single synthetic peptides were detected *ex vivo* in at least 30% of patients with RA. Since the PBMC were derived from a random group of RA patients in which 77% were taking disease modifying antirheumatic drugs, the actual number of peptide responders is probably even underestimated. Interestingly, almost 50% of the peptides (6 out of 13) that were recognized during experimental arthritis were also recognized in patients with RA. This suggests a possible similarity in the immunological process in RA patients and the experimental arthritis model in the rat. In addition, we observed that only one out of the 16 motif-based peptides that were not recognized in AA was recognized in patients with RA — this supports the idea that prescreening for identification of RA-associated autoantigens in experimental models might be useful.

The highest proliferative and RA-specific cytokine responses were found to the self-peptides derived from the MMP, MMP-1 and MMP-3. With respect to proliferation, about 50% of the RA patients responded to at least one of the 3 MMP peptides. Healthy controls showed a similar response to MMP-3 and MMP-16; however, the high percentage of responders to MMP-1 was not present in these controls. This interesting observation indicates that the proliferative response to MMP-1 is associated with RA, suggesting a role for MMP-1 in the pathophysiology of RA. The difference in proliferative response to MMP-1 between RA patients and controls might be explained by the fact that MMP-1 is absent in normal synovial tissue, but is expressed in RA, shortly after the first symptoms appear[34,35]. The observation that MMP-1 is present in synovial fluid of all

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RA patients and not in all patients with ostearthritis or post-traumatic knee injury, together with the finding that the concentration of MMP-1 in synovial fluid of RA patients is significantly higher than in other groups of knee patients, supports the hypothesis that MMP-1 plays a central role in the pathophysiology of RA[34-38]. In contrast, MMP-3 is expressed in both RA and normal synovial tissue[35]. Of note, a negative correlation between the proliferative response to MMP-3 and disease duration was observed, suggesting that RA patients lose responsiveness to MMP-3 during the course of disease. Elevated concentrations of MMP-1 and MMP-3 were found in RA synovial fluid[36-40] and serum[38,40-43], and a relation between serum MMP-3 levels and ESR, C-reactive protein, and rheumatoid factor has been reported[40-43]. Accordingly, MMP are thought to play an important role, via their proteolytic activity of structural components of the extracellular cartilage matrix, in the progressive destruction of the RA joint[44-46]. Our findings suggest an additional role of MMP, particularly MMP-1, in the pathogenesis of RA by driving autoreactive T cell responses.

The effect of the 3 MMP-derived peptides on Th1 and Th2 cell cytokine (IL-4/IFN-γ) induction was investigated, since the balance of these cytokines has been related to disease activity[18]. It was found that the production of tissue destructive cytokines (IL-1ß/TNF-α) and proteinases in rheumatoid synovitis is T cell-dependent[20]. Since the antigen-induced T cell cytokine production is very low or even undetectable, cells were additionally stimulated through CD3 and CD28. We previously demonstrated that basal and antigen-induced cytokine production by mononuclear cells correlates well with basal and antigen stimulation followed by CD3/CD28 stimulation[26-28]. Although this technique has its restrictions, at the time these studies were performed, there were no reliable alternatives. Currently we know that peptides are able to induce small amounts of IL-4 and IFN-γ detectable by multiplex cytokine analysis[47]. Because other cytokines can be detected directly, we expect that the peptides not only prime the T cells but actually induce IL-4 and IFN-γ production. We also know that when CD3/CD28 stimulation is used, only T cell cytokine production is detected[28]. We show that stimulation of RA PBMC by the MMP-1 and MMP-3 epitope induced an increase in IL-4 production in 100% and 75% of patients, respectively, concomitant with a slight reduction in IFN-γ production. This results in a decrease in the Th1/Th2 ratio, which is generally considered to be a beneficial effect in RA[18]. However, the effect on the Th1/Th2 response, calculated as the average of individual patients, was not statistically significant. Of note, IL-4 is known to inhibit MMP-1 and MMP-3 production, indicating that high IL-4 concentrations exhibit not only antiinflammatory properties[18], but also slow tissue destruction[18,48-50]. The increase in IL-4 production is correlated with disease duration, suggesting that the IL-4 response to MMP-1 and MMP-3 increases during disease development and might play a role in counteracting the disease in the long term. Thus, whereas the proliferative response to MMP-3 decreases with disease duration, the IL-4 response increases. However, before conclusions can be drawn in this respect, further studies are needed including new techniques to evaluate IL-4 production without additional manipulation, and most important, prospective followup of patients has to be performed.

MMP-3 induced an increase in both TNF-α and IL-1ß release in RA PBMC, in contrast to healthy controls. Surprisingly, in 65% of the cases the increase in TNF-α and IL-1ß coincided with an increase in IL-4, which is known to inhibit these cytokines. TNF-α and IL-1ß are suggested to be involved in cartilage degradation observed in RA. Moreover, these cytokines have been reported to increase MMP-1 and MMP-3 production[49,51-53]. In contrast to the IL-4 results, the MMP-3-induced increase in TNF-α and IL-1ß suggests a disease-promoting rather than a disease-controlling role of MMP-3-specific T cells in RA. This seeming contradiction is currently under investigation.

Irrespective of the exact role of MMP-specific T cells in the RA disease process, this study shows the presence of a specific autoimmune reaction in RA patients to MMP-1 and MMP-3-derived self-antigens, suggesting a novel role of MMP in the pathophysiology of RA. Identification of these epitopes and further characterization of the T cell responses could provide information on the role of T cells in RA and enable us to monitor RA-specific T cell responses. Moreover, MMP can become targets for antigen-specific immunotherapy, as we have shown in the AA model[54].

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REFERENCES

1. Harris ED Jr. Rheumatoid arthritis. Pathophysiology and implications for therapy. N Engl J Med 1990;322:1277-89.
2. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
3. Panayi GS, Lanchbury JS, Kingsley GH. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. Arthritis Rheum 1992;35:729-35.
4. van Eden W, van der Zee R, Taams LS, Prakken AB, van Roon J, Wauben MH. Heat-shock protein T-cell epitopes trigger a spreading regulatory control in a diversified arthritogenic T-cell response. Immunol Rev 1998;164:169-74.
5. Glant T, Csongor J, Szucs T. Immunopathologic role of proteoglycan antigens in rheumatoid joint disease. Scand J Immunol 1980;11:247-52.
6. Guerassimov A, Zhang Y, Banerjee S, et al. Cellular immunity to the G1 domain of cartilage proteoglycan aggrecan is enhanced in

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patients with rheumatoid arthritis but only after removal of keratan sulfate. Arthritis Rheum 1998;41:1019-25.
7. Londei M, Savill CM, Verhoef A, et al. Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. Proc Natl Acad Sci 1989;86:636-40.
8. Verheijden GF, Rijnders AW, Bos E, et al. Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. Arthritis Rheum 1997;40:1115-25.
9. Blass S, Schumann F, Hain NA, Engel JM, Stuhlmuller B, Burmester GR. p205 is a major target of autoreactive T cells in rheumatoid arthritis. Arthritis Rheum 1999;42:971-80.
10. Prakken AB, van Eden W, Rijkers GT, et al. Autoreactivity to human heat-shock protein 60 predicts disease remission in oligoarticular juvenile rheumatoid arthritis. Arthritis Rheum 1996;39:1826-32.
11. Albani S, Carson DA. A multistep molecular mimicry hypothesis for the pathogenesis of rheumatoid arthritis. Immunol Today 1996;17:466-70.
12. Oldstone MB. Molecular mimicry and immune-mediated diseases. FASEB J 1998;12:1255-65.
13. Leech S. Molecular mimicry in autoimmune disease. Arch Dis Child 1998;79:448-51.
14. Abu-Shakra M, Buskila D, Shoenfeld Y. Molecular mimicry between host and pathogen: examples from parasites and implication. Immunol Lett 1999;67:147-52.
15. Laskin RS. Total condylar knee replacement in patients who have rheumatoid arthritis. A ten-year follow-up study. J Bone Joint Surg Am 1990;72:529-35.
16. Alsalameh S, Mollenhauer J, Hain N, Stock KP, Kalden JR, Burmester GR. Cellular immune response toward human articular chondrocytes. T cell reactivities against chondrocyte and fibroblast membranes in destructive joint diseases. Arthritis Rheum 1990;33:1477-86.
17. Fang Q, Sun YY, Cai W, Dodge GR, Lotke PA, Williams WV. Cartilage-reactive T cells in rheumatoid synovium. Int Immunol 2000;12:659-69.
18. van Roon JA, Lafeber FPJG, Bijlsma JWJ. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis. Arthritis Rheum 2001;44:3-12.
19. Wilbrink B, Nietfeld JJ, den Otter W, van Roy JL, Bijlsma JW, Huber-Bruning O. Role of TNF alpha, in relation to IL-1 and IL-6 in the proteoglycan turnover of human articular cartilage. Br J Rheumatol 1991;30:265-71.
20. Klimiuk PA, Yang H, Goronzy JJ, Weyand CM. Production of cytokines and metalloproteinases in rheumatoid synovitis is T cell dependent. Clin Immunol 1999;90:65-78.
21. Van Bilsen JHM, Wagenaar-Hilbers JPA, Boot EPJ, van Eden W, Wauben MHM. Searching for cartilage-associated mimicry epitope in adjuvant arthritis. Autoimmunity 2002;35:201-10.
22. van Eden W, Holoshitz J, Nevo Z, Frenkel A, Klajman A, Cohen IR. Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc Natl Acad Sci USA 1985;82:5117-20.
23. Wauben MH, van der Kraan M, Grosfeld-Stulemeyer MC, Joosten I. Definition of an extended MHC class II-peptide binding motif for the autoimmune disease-associated Lewis rat RT1.BL molecule. Int Immunol 1997;9:281-90.
24. Van der Zee R, Anderton SM, Buskens CAF, Alonso de Velasco E, Van Eden W. Heat shock protein T-cell epitopes as immunogenic carriers in subunit vaccines. In: Maya HLS, editor. Peptides 1994. Proceedings of the 23rd European Peptides Symposium, Leiden, The Netherlands, 1994. Leiden: ESCOM; 1994:841-2.
25. Arnett FC, Edworthy SM, Bloch DA, et al. American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
26. Verhoef CM, Van Roon JA, Vianen ME, Glaudemans CA, Lafeber FP, Bijlsma JW. Lymphocyte stimulation by CD3-CD28 enables detection of low T cell interferon-gamma and interleukin-4 production in rheumatoid arthritis. Scand J Immunol 1999; 50:427-32.
27. van Roon JAG, van Eden W, van Roy JLAM, Lafeber FJPG, Bijlsma JWJ. Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. J Clin Invest 1997;100:459-63.
28. Verhoef CM, Van Roon JAG, Vianen ME, Bijlsma JWJ, Lafeber FPJG. Interleukin 10 (IL-10), not IL-4 or interferon-g production, correlates with progression of joint destruction in rheumatoid arthritis. J Rheumatol 2001;28:1960-6.
29. Maurice MM, Lankester AC, Bezemer AC, et al. Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis. J Immunol 1997;159:2973-8.
30. Matsuda M, Ulfgren AK, Lenkei R, et al. Decreased expression of signal-transducing CD3 zeta chains in T cells from the joints and peripheral blood of rheumatoid arthritis patients. Scand J Immunol 1998;47:254-62.
31. Malmstrom V, Kjellen P, Holmdahl R. Type II collagen in cartilage evokes peptide-specific tolerance and skews the immune response. J Autoimmun 1998;11:213-21.
32. Corthay A, Backlund J, Broddefalk J, et al. Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. Eur J Immunol 1998;28:2580-90.
33. Kim HY, Kim WU, Cho ML, et al. Enhanced T cell proliferative response to type II collagen and synthetic peptide CII (255-274) in patients with rheumatoid arthritis. Arthritis Rheum 1999; 42:2085-93.
34. Konttinen YT, Ainola M, Valleala H, et al. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis 1999;58:691-7.
35. Cunnane G, FitzGerald O, Hummel KM, Gay RE, Gay S, Bresnihan B. Collagenase, cathepsin B and cathepsin L gene expression in the synovial membrane of patients with early inflammatory arthritis. Rheumatology 1999;38:34-42.
36. Clark IM, Powell LK, Ramsey S, Hazleman BL, Cawston TE. The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1993;36:372-9.
37. Walakovits LA, Moore VL, Bhardwaj N, Gallick GS, Lark MW. Detection of stromelysin and collagenase in synovial fluid from patients with rheumatoid arthritis and posttraumatic knee injury. Arthritis Rheum 1992;35:35-42.
38. Sasaki S, Iwata H, Ishiguro N, Obata K, Miura T. Detection of stromelysin in synovial fluid and serum from patients with rheumatoid arthritis and osteoarthritis. Clin Rheumatol 1994;13:228-33.
39. Cawston TE, Mercer E, de Silva M, Hazleman BL. Metalloproteinases and collagenase inhibitors in rheumatoid synovial fluid. Arthritis Rheum 1984;27:285-90.
40. Yoshihara Y, Obata K, Fujimoto N, Yamashita K, Hayakawa T, Shimmei M. Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. Arthritis Rheum 1995;38:969-75.
41. Manicourt DH, Fujimoto N, Obata K, Thonar EJ. Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. Relationship to serum levels of antigenic keratan sulfate and systemic parameters of inflammation. Arthritis Rheum 1995;38:1031-9.

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42. Keyszer G, Lambiri I, Nagel R, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. J Rheumatol 1999; 26:251-8.
43. Cunnana G, Fitzgerald O, Beeton C, Cawston TE, Bresnihan B. Early joint erosions and serum levels of matrix metalloproteinase 1, matrix metalloproteinase 3, and tissue inhibitor of matrix metalloproteinase 1 in rheumatoid arthritis. Arthritis Rheum 2001;44:2263-74.
44. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 1993; 4:197-250.
45. Johnson LL, Dyer R, Hupe DJ. Matrix metalloproteinases. Curr Opin Chem Biol 1998;2:466-71.
46. Vincenti MP, Clark IM, Brinckerhoff CE. Using inhibitors of metalloproteinases to treat arthritis. Easier said than done? Arthritis Rheum 1994;37:1115-26.
47. De Jong H, de Jager W, Taams LS, et al. Novel pan DR binding HSP60 peptides induce CD4 positive T cells producing TNFa, IL-1ß and IL-10 in rheumatoid arthritis [abstract]. Arthritis Rheum 2002;45 Suppl:S487.
48. Nemoto O, Yamada H, Kikuchi T, et al. Suppression of matrix metalloproteinase-3 synthesis by interleukin-4 in human articular chondrocytes. J Rheumatol 1997;24:1774-9.
49. Borghaei RC, Rawlings PL Jr, Mochan E. Interleukin-4 suppression of interleukin-1-induced transcription of collagenase (MMP-1) and stromelysin 1 (MMP-3) in human synovial fibroblasts. Arthritis Rheum 1998;41:1398-406.
50. Lubberts E, Joosten LA, van den Bersselaar L, et al. Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. J Immunol 1999;163:4546-56.
51. MacNaul KL, Chartrain N, Lark M, Tocci MJ, Hutchinson NI. Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-alpha on stromelysin expression. J Biol Chem 1990;265:17238-45.
52. McCachren SS, Greer PK, Niedel JE. Regulation of human synovial fibroblast collagenase messenger RNA by interleukin-1. Arthritis Rheum 1989;32:1539-45.
53. Mizel SB, Dayer JM, Krane SM, Mergenhagen SE. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin 1). Proc Natl Acad Sci USA 1981;78:2474-7.
54. Van Bilsen JHM, Wagenaar-Hilbers JPA, van der Cammen MJF, van Dijk MEA, van Eden W, Wauben MHM. Successful immunotherapy with MMP-derived peptides in adjuvant arthritis depends on timing of peptide administration. Arthritis Res 2002;4:R2.

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