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Chemokine expression in rheumatoid arthritis (RA): evidence of RANTES and macrophage inflammatory protein (MIP)-1β production by synovial T cells

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SUMMARY

Earlier studies from this laboratory provided evidence for restricted cytokine expression in the T cell population in RA tissues. Specifically, IL-2, IL-4, IL-6 and interferon-gamma (IFN-γ) gene expression levels were low. The selective chemoattractant and activation effects of chemokines on leucocytes identify them as potentially ideal candidates in mediating selective inflammatory processes in RA. Accordingly, we undertook studies to examine constitutive chemokine gene expression in RA tissues. RANTES, monocyte chemotactic protein-1 (MCP-1) and MIP-1β gene expression was examined in both the T and non-T cell populations in RA peripheral blood (PB), synovial fluid (SF) and synovial tissues (ST). Our results identified elevated levels of both RANTES and MIP-1β gene expression in circulating RA PB and SF T cells. By contrast, MCP-1 expression was virtually absent in RA PB, yet elevated MCP-1 mRNA levels were detected primarily in the non-T cell populations of the SF and ST samples. Histological examination of affected rheumatoid joints revealed extensive RANTES and MIP-1β expression in sites of lymphocyte infiltration and cell proliferation, namely the synovial lining and sublining layers. Fractionation of RA ST patient samples revealed that RANTES expression was restricted to the T cells, whereas MIP-1β expression was detected in both T and non-T fractions. These data suggest that MCP-1, MIP-1β and RANTES may have a central role in the trafficking of reactive molecules involved in immunoregulation and in the inflammatory processes in RA.

Keywords chemokine rheumatoid arthritis

INTRODUCTION

RA is an inflammatory joint disease, characterized by disordered immune regulation, in which joint destruction results from the unopposed degradative activities of pro-inflammatory cytokines [1–10]. The biology of RA is complicated, since the synovial environment is an area of intense immunological activity. The cellular composition of the inflamed synovial tissues (ST) reveals an extensive infiltrate of T cells and macrophages, together with synovial fibroblasts. The fibroblast and mononuclear cells secrete predominantly pro-inflammatory and proliferative cytokines, important in mediating the pathophysiologic events in RA. Accumulated data implicate a restricted T cell cytokine profile in RA which may limit normal immunoregulatory functions necessary to attenuate the chronic synovitis characteristic of this disease [11–23]. Failure to detect significant levels of interferon-gamma (IFN-γ) and IL-2 in the RA synovium is particularly suprising, given the state of activation and $CD4^+/45RO^+$ phenotype of the T cells within the synovium [24–31]. The factors responsible for the restricted production of T cell-derived cytokines relative to the up-regulated production of other cytokines in the RA synovium are unknown.

Chemokines, a superfamily of structurally related cytokines, exhibit leucocyte attractant and activating properties (reviewed in [32]): monocyte chemotactic protein 1 (MCP-1) is, as its name implies, a chemoattractant for monocytes and regulates adhesion molecule and cytokine expression in these cells [33–35]. RANTES is selectively chemoattractant for $CD45RO^+$ memory T cells [36], and recent reports have detailed that MIP-1β preferentially attracts $CD4^+$ T cells [37,38]. The $CD4^+/45RO^+$ T cells, as indicated above, are prevalent in the RA synovium. The selective chemoattractant and pro-adhesive effects of chemokines identify them as candidates to play a key role in lymphocyte trafficking in RA. Indeed, recent reports have shown that inflamed, hypertrophic rheumatoid ST contains cells that express the MCP-1 gene, and cultured rheumatoid synoviocytes produce MCP-1 in response to IL-1, tumour necrosis factor-alpha (TNF-α), platelet-

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derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), factors that are known to be present in synovitis [39–44]. Moreover, the expression of RANTES may be induced by IL-1 and TNF-α in cultured rheumatoid synovial fibroblasts [45].

Since an earlier report from this laboratory demonstrated that RA tissues do not exhibit elevated levels of IL-2, IL-4, IL-6 and IFN-γ in their T cells [21], the present study was undertaken to examine RA tissues for constitutive gene expression for MIP-1β and RANTES, again in the T cell fractions. RA peripheral blood (PB), synovial fluid (SF) and ST samples were analysed. In parallel, we determined gene expression levels for these cytokines and MCP-1 in the non-T cell fractions derived from the same RA tissues. Our results clearly demonstrate the presence of MIP-1β and RANTES in circulating RA PB. Whereas MIP-1β gene expression was detected in both the T and non-T cell fractions, RANTES expression was present in the T cell fraction alone. Of note, MCP-1 gene expression was virtually absent in all the RA PB samples examined. Examination of the SF and ST of affected RA joints revealed elevated expression levels for MIP-1β, RANTES and MCP-1, with the majority of MCP-1 expressed in the non-T cell fractions isolated from ST. These data implicate specific chemokines in lymphocyte trafficking to the affected rheumatoid joint, and suggest a potential role for these chemokines in mediating the persistent synovitis in RA.

PATIENTS AND METHODS

Patient selection

Sixty patients with seropositive RA according to the American College of Rheumatology criteria [46], seen at the Rheumatic Disease Unit and Orthopaedic Service at the Wellesley Hospital, Toronto, were included in the study. The control group ($n = 6$) consisted of age-matched healthy volunteers. Most patients were receiving a non-steroidal anti-inflammatory drug and a remittive agent. No patient was receiving > 5 mg prednisone/day.

Preparation of ST, SF and PB mononuclear cells

ST obtained at the time of surgical synovectomy was treated for 1·5 h with DNAse and collagenase in PBS with 1% bovine serum albumin (BSA) at 37°C. The resultant cell suspension was strained through sterile gauze, then washed with PBS. SF was aspirated into heparinized syringes. SF and PB were diluted 1 : 1 with PBS.

Mononuclear cells were isolated by density centrifugation on a Ficoll–Hypaque gradient at 500 ***g*** for 45 min, then washed twice with PBS in preparation for T and non-T cell separations. All cell preparations were processed immediately.

Mitogenic activation of HL-60 (promyelocytic leukaemia) cells

HL-60 cells (10^6/ml) were stimulated with 50 ng/ml phorbol myrsitate acetate (PMA; phorbol 12-myristate 13-acetate) in 20% RPMI 1640. The cells were incubated for up to 24 h at 37°C in 5% CO_2 and mRNA was extracted at various time points.

T and non-T cell fractionation

Mononuclear cells were fractionated into T and non-T cell preparations by separation of T cells using a standard rosetting protocol that routinely yielded > 90% T cells. Briefly, following rosetting with AET-treated sheep erythrocytes and centrifugation on a Ficoll–Hypaque gradient at 800 ***g*** for 45 min, the T and non-T cell fractions separated: T cells rosetted to the sheep erythrocytes in the lower layer, whereas the non-T cell fraction migrated to the interface between the layers. Sheep erythrocytes were removed by lysing in an NH_4Cl–Tris buffer at 37°C for 5 min. T cells were then washed twice with PBS, lysed in 4 M guanidinium isothiocyanate, and either used immediately or stored at −70°C. Similarly, the non-T cell fraction was washed in PBS, lysed and used immediately or stored at −70°C.

RNA isolation

RNA extraction from mononuclear cells has been described [47]. Briefly, following lysis with guanidinium isothiocyanate, nucleic acid was extracted from cells with phenol-chloroform and the RNA precipitated with ethanol. Poly A+ RNA was separated by oligo-dT cellulose and precipitated for 2 h at −70°C with 2 M Na actetate and ethanol.

cDNA preparation

Poly A+ RNA from 0·5 μg/ml total RNA was reverse transcribed with 200 U Moloney murine leukaemia virus reverse transcriptase, according to standard procedures [48]. The cDNA (20 μl) was then used immediately in a polymerase chain reaction (PCR).

PCR amplification

Each PCR reaction was carried out in a total volume of 50 μl, containing the cDNA (20 μl), 50 mM KCl, 10 mM Tris pH 8·3, 1·5 mM $MgCl_2$, 0·01% gelatin, 0·2 mM each of dATP, dCTP and dGTP, 2·5 U Taq polymerase, 0·4 μM of each specific primer and 10 μCi of ^{32}P-dTTP. Amplification of individual cytokines was carried out in separate tubes. Each reaction tube was overlaid with 50 μl mineral oil. Thirty cycles of amplification were performed, each cycle consisting of a denaturation step at 94°C for 1 min, annealing at 58°C for 2 min and polymerization at 72°C for 3 min. In the final cycle, polymerization was for 7 min.

The following chemokine sense and antisense primers were used: MCP-1, 5′-CCAATTCTCAAACTGAAGCTCG-CAC-3′ and 5′-CTTAGCTGCAGATTCTTGGGTTGTG-3′; RANTES, 5′-ATGAAGGTCTCCGCGGCAGCCC-3′ and 5′-CTAGCTCATCTCCAAAGAGTTG-3′; MIP-1β, 5′-ACCAT-CAAGCTCTGCGTGACTG-3′ and 5′-GCAGGTCAGTT-CAGTTCCAGGTC-3′.

Electrophoresis and autoradiography

Following PCR amplification, each sample was fractionated on a separate Sephadex G50 spin column to remove unincorporated radionucleotides and 15-μl aliquots were electrophoresed on a 1% agarose gel at 55 V for 2 h. A Hind III-digested λ marker was included in each run. The identity of the amplified products was confirmed by their specific respective sizes. The agarose gels were soaked in 7% trichloroacetic acid for 30 min, then dried. The dried gel was then exposed to Kodak XAR-5 x-ray film (Eastman-Kodak, Rochester, NY). The size of the autoradiographic signal was quantified using laser densitometry, and all values were normalized against the standard positive control signals.

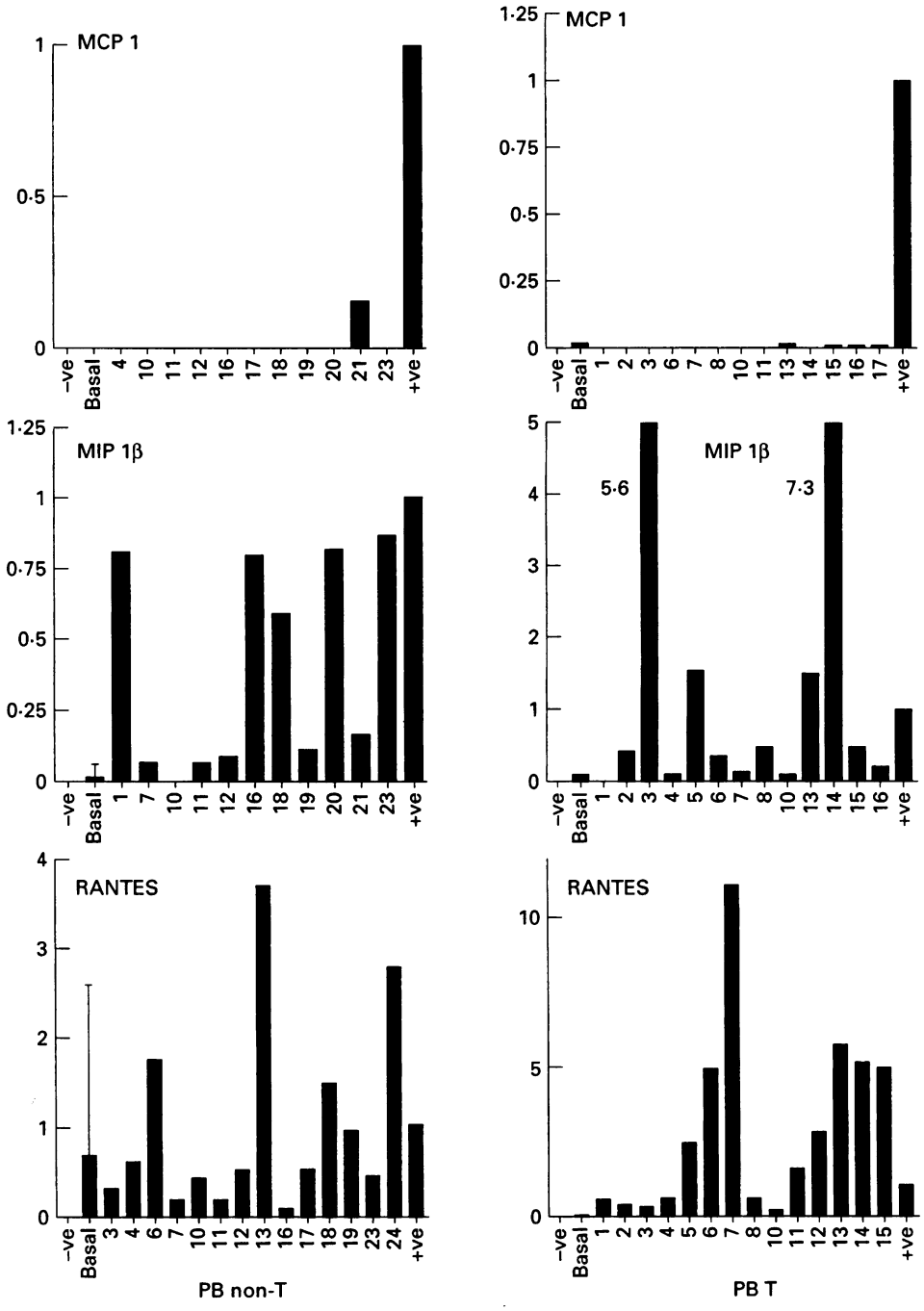

Fig. 1. Quantification of chemokine gene expression in peripheral blood (PB) T and non-T cell samples from patients with RA. Chemokine gene expression, as indicated in the individual panels, was quantified by polymerase chain reaction (PCR) amplification of cDNAs derived from isolated poly A + mRNA. All values are quantified relative to the positive control (+ ve) included in each PCR determination, normalized to a value of 1. Basal, the baseline level of mRNA expression determined from unstimulated PB T and non-T cells from healthy donors (mean and s.d. of six different samples). Each number identifies a particular patient.

In situ *hybridization*

ST were snap frozen in dry ice/2-methy-butane and stored at −70°C until use. Frozen sections (7 μm) were cut, mounted onto slides, fixed in 4% paraformaldehyde, treated with proteinase K (PK), then transferred to 0·5 × SSC. Hybridization was carried out with 10^4 ct/min per μl of antisense probe. For RANTES, the probe consisted of an antisense transcript from bp 1 to 410, comprising the entire coding region, as well as some 5′ and 3′ untranslated nucleotides [49]. For MIP-1β, a cDNA representing the full length mRNA, devoid of the polyA tail, was used [36]. Both cDNAs were cloned into a plasmid in an antisense orientation relative to the T7 promoter, from which antisense nucleotide probes were genereated incorporating ^{35}S dUTP, using standard *in vitro* transcription techniques. Hybridizations were performed in 50% formamide, 2 × SSC, 500 μg/ml tRNA and 10% dextran sulphate, overnight at 55°C.

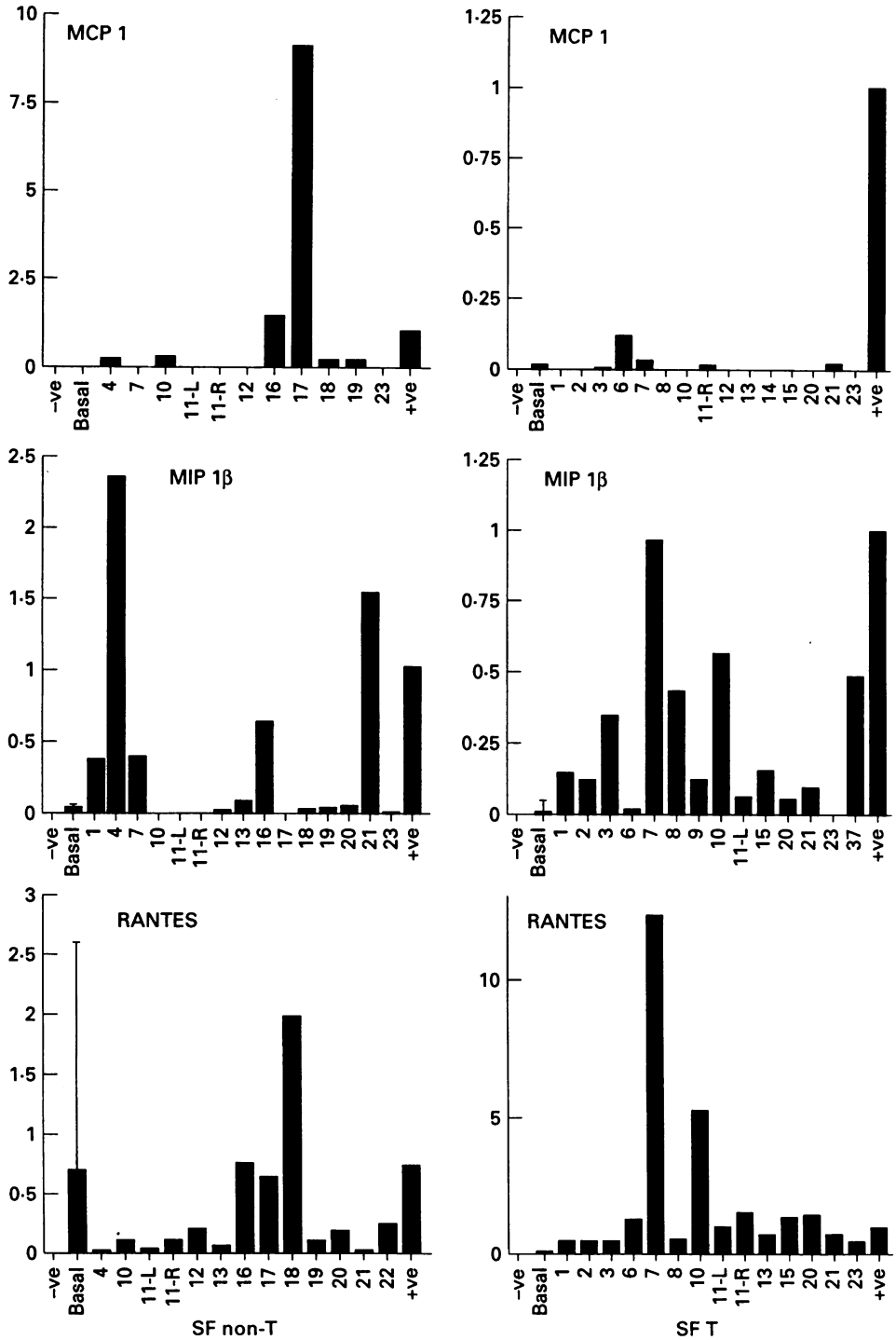

Fig. 2. Quantification of chemokine gene expression in synovial fluid (SF) T and non-T cell samples from patients with RA. Chemokine gene expression, as indicated in the individual panels, was quantified by polymerase chain reaction (PCR) amplification of cDNAs derived from isolated poly A+ mRNA. All values are quantified relative to the positive control (+ ve) included in each PCR determination, normalized to a value of 1. Basal, the baseline level of mRNA expression determined from unstimulated peripheral blood (PB) T and non-T cells from healthy donors (mean and s.d. of six different samples). Each number identifies a particular patient. Patient numbers, where appropriate, are consistent with those in Fig. 1. 11-R and 11-L denote samples taken from patient no. 11, either from the right knee or left knee joints, respectively.

After extensive washing, the specimens were dehydrated, air-dried and covered with Kodak NTB nuclear track emulsion for autoradiography. After exposure at 4°C, the slides were developed, fixed and counterstained with haematoxylin and eosin. As negative controls, replicate slides were hybridized with RANTES or MIP-1β sense probes.

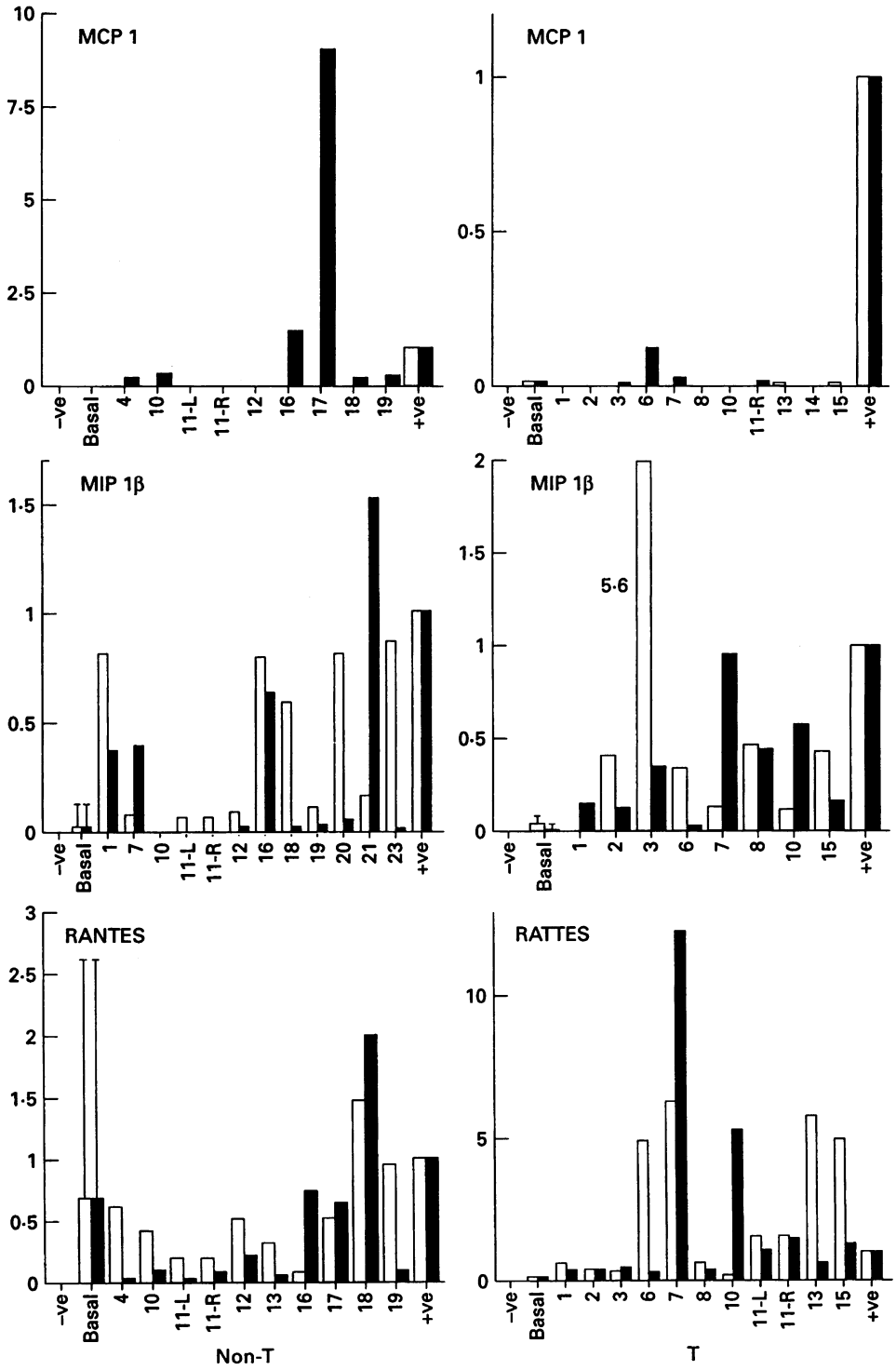

Fig. 3. Quantification of chemokine gene expression in matched peripheral blood (PB, □) and synovial fluid (SF, ■) T and non-T cell samples from patients with RA. Chemokine gene expression, as indicated in the individual panels, was quantified by polymerase chain reaction (PCR) amplification of cDNAs derived from isolated poly A+ mRNA. All values are quantified relative to the positive control (+ ve) included in each PCR determination, normalized to a value of 1. Basal, the baseline level of mRNA expression determined from unstimulated PB T and non-T cells from healthy donors (mean and s.d. of six different samples). Each number identifies a particular patient. Patient numbers, where appropriate, are consistent with those in Fig. 1. 11-R and 11-L denote samples taken from patient no. 11, either from the right knee or left knee joints, respectively.

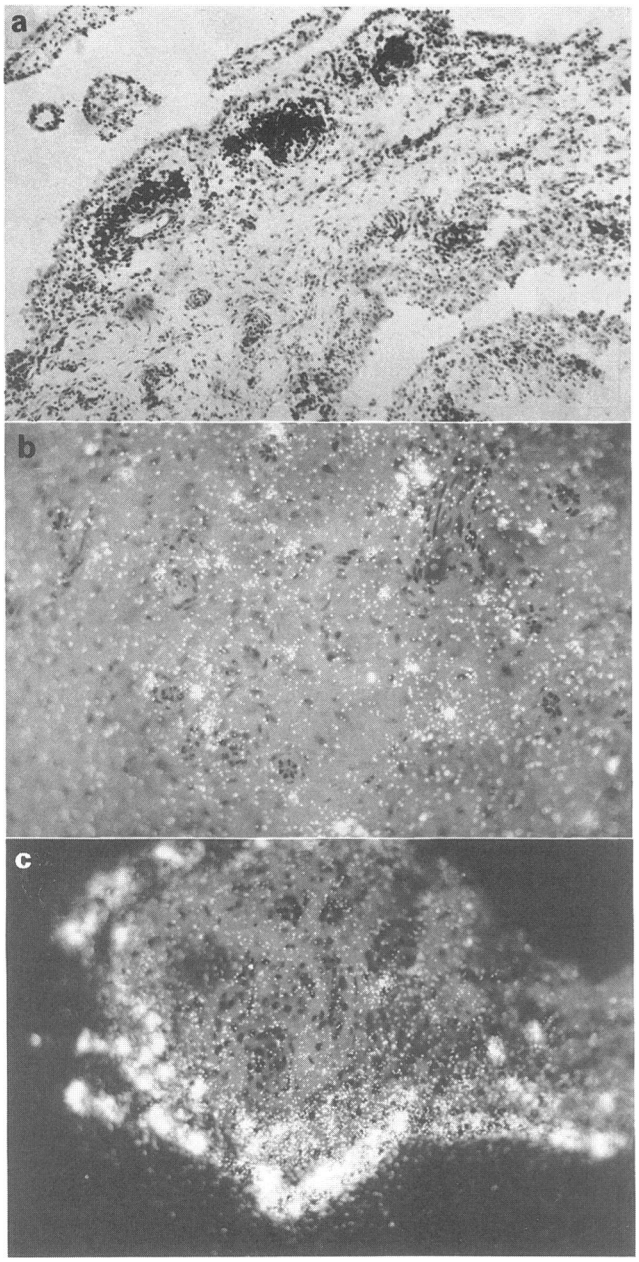

Fig. 4. *In situ* hybridization of antisense probes for MIP-1β and RANTES to frozen human RA synovial tissues (ST). For experimental details refer to Patients and Methods. (a) A representative micrograph illustrating anti-Leu-4 staining of lymphoid aggregates that are predominantly composed of T cells. Other scattered T cell infiltration is also detected. (b,c) RANTES and MIP-1β antisense probe hybridization (bright grains) among mononuclear cell agregates, respectively.

RESULTS

Chemokine expression by PMA-activated HL-60 cells

Initially, studies were conducted to determine and compare the kinetics of induction and accumulation of RANTES, MIP-1β and MCP-1 mRNAs, following PMA activation of HL-60 cultures. In time course studies we quantified chemokine mRNA expression using the PCR protocol. The results of replicate experiments demonstrated that MIP-1β was maximally induced by 4 h following PMA stimulation of HL-60 cultures, MCP-1 by 8 h and RANTES by 24 h (data not shown). In all subsequent experiments the time point which yielded maximal expression for a particular chemokine served as the positive control, normalized to a value of 1, and all patient samples were quantified relative to the positive control.

An earlier study from this laboratory reported that the tissue-processing protocol, which we employed for the present study, had no effect on the composition or integrity of the RNA recovered from cells, although a reduction in viable cell yield was recorded at the different stages [21]. Specifically, 3–5 μg RNA/10^6 cells were consistently recovered from resultant viable cells at each stage of the purification procedure.

Chemokine mRNA expression in RA PB and SF T and non-T fractions

The results in Figs 1 and 2 show mRNA expression levels for MCP-1, MIP-1β and RANTES in RA PB and SF samples, respectively. Despite the variability in levels of chemokine gene expression among patient samples, only values > 2 s.d. above mean basal levels were considered significant. Similar outcomes with regard to gene expression levels were obtained for randomly selected patient samples that were analysed repeatedly (three times) during different 'test' runs (data not shown), confirming the reproducibility of our results. The positive PMA-activated HL-60 control was included for each chemokine in each of the PCR amplifications. A baseline control, determined from unstimulated PB T and non-T cell fractions obtained from six healthy donors, was included for comparison. Although each 'test' run included a positive control that was normalized to a value of 1·0, the signal intensity of this positive control band remained uniform across experiments. Moreover, the inclusion of β-actin primers to amplify a reporter gene product in randomly selected patient samples confirmed both accurate measurement of and the integrity of the mRNA.

In only one of the RA PB preparations we examined did we detect any MCP-1 gene expression (Fig. 1). MIP-1β gene expression was elevated in both the T and non-T cell fractions in the RA PB patient samples screened. RANTES gene expression was present in both the non-T and T cell fractions of the RA PB patient samples, yet, based on the baseline gene expression levels detected in the healthy controls, was significantly elevated predominantly in all 14 of the RA PB T cell fractions.

Figure 2 shows a similar analysis of data derived from the RA SF patient samples. MCP-1 gene expression was essentially absent in the SF T cell fractions, yet 6/11 patient samples demonstrated significantly elevated gene expression levels in the non-T cell fraction of the SF. As seen in the PB samples, MIP-1β gene expression levels were elevated in both the T (12/14) and non-T (6/15) cell fractions. RANTES gene expression was detectable in both the T and non-T cell fractions, but was only significantly elevated above basal levels in the T cell samples, where all the samples tested exceeded those of the PB of healthy controls.

In Fig. 3 the data have been collated to indicate the chemokine expression levels in paired SF and PB derived from RA patients. As noted above, MCP-1 gene expression was virtually absent in all of the RA PB samples examined, yet six of the nine corresponding paired RA SF non-T cell samples

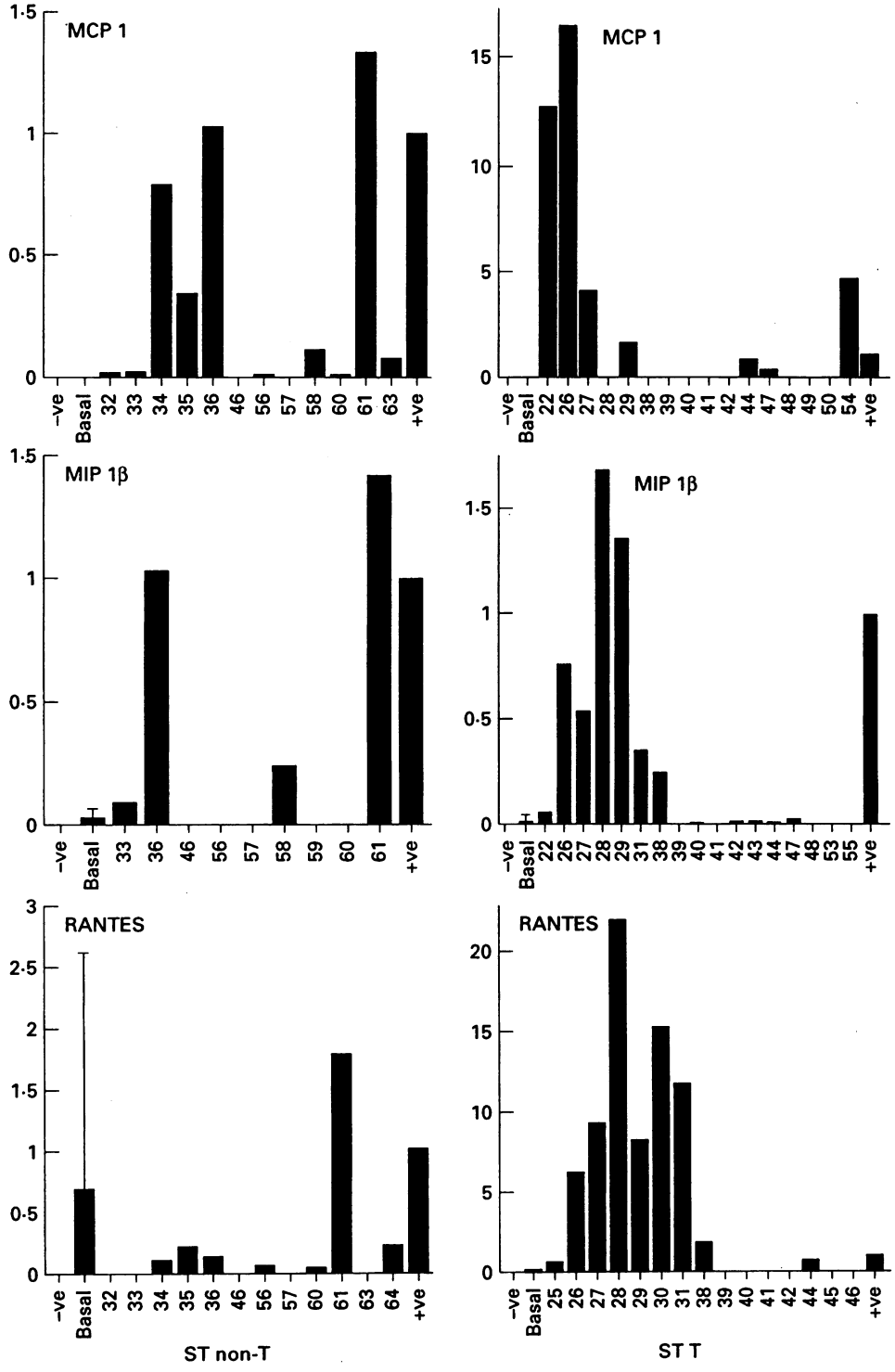

Fig. 5. Quantification of chemokine gene expression in RA synovial tissue (ST) T and non-T cells. Chemokine gene expression, as indicated in the individual panels, was quantified by polymerase chain reaction (PCR) amplification of cDNAs derived from isolated poly A + mRNA. All values are quantified relative to the positive control (+ ve) included in each PCR determination, normalized to a value of 1. Basal, the baseline level of mRNA expression determined from unstimulated peripheral blood (PB) T and non-T cells from healthy donors (mean and s.d. of six different samples). Each number identifies a particular patient. Patient numbers, where appropriate, are consistent with those in Fig. 1.

Table 1. Summary of chemokine expression in RA

Fraction	Compartment	MCP-1	MIP-1β	RANTES
T cells	PB	0/13 (0)	12/13 (92)	14/14 (100)
	SF	2/15 (13)	12/14 (86)	14/14 (100)
	ST	7/16 (44)	7/17 (41)	9/15 (60)
Non-T cells	PB	1/11 (9)	10/11 (91)	2/14 (14)
	SF	6/11 (55)	6/15 (40)	0/13 (0)
	ST	10/12 (83)	4/9 (44)	0/12 (0)

Values $\geqslant$ 2 s.d. above mean of baseline levels determined from unstimulated peripheral blood (PB) from six healthy donors, are recorded. Values given in parentheses are the per cent of positive patient samples.

SF, Synovial fluids; ST, synovial tissue; MCP-1, monocyte chemotactic protein-1.

examined exhibited elevated expression for this chemokine. The implications are that MCP-1 is produced locally at the site of inflammation and is not sequestered into the circulation. By contrast, with few exceptions, MIP-1β gene expression was detected in both the PB and SF compartments of the RA patients. Similarly, RANTES gene expression was evident in all of the matched T cell PB and SF patient samples. For each of the chemokines examined, the level of mRNA expression in the PB of a patient did not necessarily correlate with the expression level detected in the matched SF.

Chemokine mRNA expression in RA ST

Having identified that RA SF cells expressed mRNA for MCP-1, MIP-1β and RANTES, we extended our investigations to include RA ST. At the outset we examined thin sections of ST for RANTES and MIP-1β gene expression by *in situ* hybridization with the antisense probes specific for the individual chemokines. The results are shown in Fig. 4. Figure 4a is a representative micrograph illustrating anti-Leu-4 staining of lymphoid aggregates that are predominantly composed of T cells. Other scattered T cell infiltration is also detected. Figure 4b,c depict the RANTES and MIP-1β antisense probe hybridization (bright grains) among mononuclear cell aggregates. Of note is the intense staining in the synovial lining layer, characterized histologically in RA by infiltration of leucocytes and by marked hyperplasia.

In subsequent experiments we examined mRNA expression for RANTES, MIP-1β and MCP-1 in RA ST T and non-T cell samples, using the PCR protocol. The results shown in Fig. 5 indicate that 10 of the 12 non-T cell fractions and seven of the 16 T cell fractions, expressed elevated levels of MCP-1 mRNA expression. Although some consideration must be given to the low-level monocyte contamination of fractionated T cells that would account for some MCP-1 expression, there are two observations that suggest that this is not a significant confounding factor: (i) MCP-1 gene expression was detected in only 2/15 SF T cell patient samples (Fig. 2), that would similarly be subject to low-level non-T cell contamination; and (ii) expression levels recorded were considerably lower ($\leqslant$ 0·1) in the SF T cell samples that exhibited MCP-1 expression than in the ST T cell patient samples (Fig. 5, 0·35–16·3). Moreover, accumulating evidence suggests that MCP-1 expression is inducible in a wide variety of tissues [36], despite the restricted target sensitivity of MCP-1 for monocytes and basophils. Consistent with the *in situ* hybridization data, we detected elevated expression for MIP-1β in both T and non-T cell fractions and RANTES in the T cell fractions alone. Randomly selected patient samples were again analysed repeatedly in different 'test' runs, to confirm the utility of the protocol. As

Table 2. Restricted cytokine expression in RA synovial T cells

Patient*	Tissue	IL-2	IL-4	IL-6	IFN-γ	RANTES	MIP-1β	MCP-1
7	SFT	B	–	–	0	12·3	0·96	0·03
10	SFT	0	0	0·8	0	5·3	0·57	0
3	SFT	0·4	0	1·2	0	0·46	0·34	0·01
8	SFT	0·4	–	–	0	0·4	0·44	0
1	SFT	0·94	B	0	0	0·41	0·15	B
2	SFT	B	–	–	0	0·43	0·12	0
23	SFT	B	0	0	0	0·48	0	0
6	SFT	0	–	–	0	1·21	0·02	0·12
21	SFT	B	–	–	0	0·72	0·09	0·01
20	SFT	B	–	–	0	1·37	0·05	0
11	SFT	B	–	–	0	1·01	0·06	0·01
27	STT	0	0	0	–	9·2	0·53	16·34
26	STT	B	B	4·0	–	6·23	0·76	12·56
53	STT	B	–	–	0	–	0	4·0

* Patient numbers correspond to those in Figs 2, 3 and 5.

B, The baseline level of mRNA expression determined from unstimulated peripheral blood (PB) T cells from healthy donors (mean and s.d. of six different samples). Cytokine mRNA expression levels are quantified relative to the positive control included in each polymerase chain reaction (PCR) determination (see Patients and Methods), that was normalized to a value of 1·0. The results for IL-2, IL-4, IL-6 and IFN-γ have been reported earlier [21]. All values above 0 are > 2 s.d. above B.

– denotes 'not done'.

MCP-1, Monocyte chemotactic protein-1.

before, similar results were obtained across experiments, for both the positive controls and test samples.

DISCUSSION

Table 1 summarizes the data collected for all the RA patient tissues. Unfortunately, the scope of this study, together with the limited availability of patient material, precluded examination of each patient sample, whether it was PB, SF or ST, for each of the chemokines under investigation, the more so since the intention was to perform replicate amplifications on the patient samples. Although considerable variation was seen in the expression levels for the different chemokines among the different patient tissues, a number of trends with regard to chemokine gene expression in RA tissues is emerging. RA PB mononuclear cells express both MIP-1β and RANTES mRNA, the expression of MIP-1β being relatively evenly distributed between the T and non-T cell fractions, and elevated RANTES mRNA expression essentially restricted to the T cell fraction of PB mononuclear cells. Moreover, the patterns of expression for MIP-1β and RANTES within the inflamed RA synovium reflect that observed in the PB. In none of the 13 PB T and only one of the 11 non-T cell fractions was MCP-1 mRNA detected. However, there is good evidence for MCP-1-producing cells in both the SF and ST non-T cell populations. The implications are that MCP-1 is produced at the site of joint inflammation, whereas both MIP-1β- and RANTES-expressing cells are found both locally and systemically.

Accumulating evidence implicates the infiltrating mononuclear phagocytes in orchestrating immune and inflammatory responses within the rheumatoid joint, through their ability to produce multiple regulatory factors. An unanswered question has been the role of the phenotypically activated infiltrating T cell in mediating the persistent inflammation in the RA joint. We have previously reported that these reactive T cells exhibit a restricted cytokine profile, specifically in regard to IL-2, IL-4, IL-6 and IFN-γ. Our present data suggest that the restricted production of the above mentioned cytokines may reflect T cells that are committed to pathogenic events associated with chemokine production. The data presented in Table 2 support the notion of RA T cells in the rheumatoid joint exhibiting restricted cytokine expression. There is clear evidence for SF T cells expressing MIP-1β and RANTES, in the absence of IL-2 and IFN-γ. It is intriguing to speculate that the chemokines identified in the RA tissues in this study may influence the overall pattern of cytokine production in the rheumatoid tissues, in part by down-regulating the expression of specific T cell-secreted cytokines such as IL-2 and IFN-γ.

The selective chemoattractant and pro-adhesive effects of MIP-1β and RANTES for T cells and MCP-1 for macrophages, identifies them as likely candidates orchestrating the lymphocyte trafficking in RA. Indeed, our *in situ* hybridizations identified prominent staining for RANTES and MIP-1β in the synovial lining layer and sublining regions of the rheumatoid synovium, sites of infiltration of reactive lymphocytes in affected RA joints. The ability of these chemokines to mediate the destructive activities of the infiltrating inflammatory cells, by activating or regulating specific subsets of lymphocytes, is a subject for further investigation. Moreover, the involvement of other chemokines in this inflammatory disease remains to be addressed. Certainly, the evidence presented here suggests a role for chemokines in the pathogenesis and persistence of inflammation and joint destruction in RA.

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