Table I. Comparison of allelic frequency between rheumatoid arthritis (RA) patients and control subjects.

	Alleles frequency			Carriage rate		
	RA	Controls	P	RA	Controls	P
IL4 Intron 3						
RP1	173 (83.2)	165 (80.1)	NS	101 (97)	99 (96.1)	NS
RP2	34 (16.3)	41 (19.9)	NS	31 (29.8)	37 (35.9)	NS
RP0	1 (0.5)	0	NS	1 (0.9)	0	
IL-4 promoter						
C	30 (14.4)	44 (21.4)	NS	27 (26)	38 (35.8)	NS
T	178 (85.6)	162 (78.6)	NS	101 (97.1)	97 (94.2)	NS

*NS: not significant.

tween RA patients and control populations. As shown in table, no significant associations were observed in the distribution of genotypes, cytokine allele frequencies, and carriage rates between patients with RA and healthy controls. Furthermore, no statistical association in the distribution of IL-4 gene polymorphism frequency between RF positive and RF negative patients was observed.

In our present study, the distribution of IL-4 genotype at intron 3 (RP1/RP1 64.1%,RP1/RP2 32% and RP2/RP 23.9%) and promoter (C/C 5.8%, C/T 31.1%, and T/T 63.1%) in healthy Taiwanese subjects was different with data reported by Cantagrel et al. (5) in French Caucasian controls (intron 3 RP1/RP1 0%, RP1/RP2 17.9% and RP2/RP2 82.1% and promoter C/C 69.5%, C/T 30.5%, and T/T 0%, respectively). Recently, Cantagrel *et al.* demonstrated that the RP1 allele of the IL-4 gene was found a significantly higher frequency in French Caucasian RA patients compared with controls (5). In addition, the independent OR for IL-4 RP1 and IL-4 –590* T alleles in RA susceptibility were both much lower than the OR for carriage of both of these alleles together. While RA patients positive for the RP2 allele was found associated with less joint destruction in another report by Buchs *et al.* (6). In our present study, we did not observe any association in the distribution of IL-4 (intron 3 and promoter) genotypes, cytokine allele frequencies, and carriage rates between patients with RA and healthy controls. Although the frequency of the genotype of IL-4 promoter C/T in the RF positive patients was higher than that of the RF negative patients,the association did not reach significant difference.

In summary, the polymorphisms in the IL4 gene (intron 3 and promoter) studied here did not reveal any association with an increased risk of developing RA in Taiwan Chinese when compared with the control group. These results may be due to ethnic factors and will need further confirmation.

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Melatonin in rheumatoid arthritis: A disease-promoting and modulating hormone?

Sirs,

An altered functioning of the hypothalamic-pituitary-adrenal/gonadal axis seems to be an important factor in the perpetuation and circadian symptoms of rheumatoid arthritis (RA) (1). As a matter of fact, the clinical symptoms of RA show a circadian variation with joint stiffness and pain being more prominent in the early morning. Consistently, human pro-inflammatory cytokine production exhibits a diurnal rhythmicity with peak levels during the night and early morning at a time when plasma cortisol is lowest (2). The existence of a causal relationship between plasma cortisol and production of inflammatory cytokines is suggested by the finding that administration of cortisone acetate at physiological doses results in a corresponding reduction in pro-inflammatory cytokine production (2). An inappropriate low secretion of cortisol is a further typical feature of the inflammatory disease in patients with RA (1). Similarly, the secretion of adrenal androgens is significantly reduced (1).

However, cortisol may not be the only hormone affecting cytokine rhythms; a strong candidate is the pineal indoleamine melatonin, the circadian hormone “par excellence”, whose synthesis and secretion is regulated by the photoperiod with peak levels during the night,darkness hours (3). The circadian nocturnal release of melatonin has a profound influence on the internal environment of the organism with diverse physiological effects. Its main function seems to be that of synchronising the organism in the photoperiod and may play a role in reproduction, metabolism, seasonality, thermoregulation and immunity (4). In peripheral blood mononuclear cells, melatonin has been reported to stimulate the production of interleukin-2 interferon-gamma and interleukin-6 but not that of interleukin-4 (5). Physiologically, the nocturnal MLT peak has been associated with high IFN-γ/interleukin-10 ratio, i.e. the melatonin rhythm positively correlated with the rhythmicity of T-helper cell type 1/ T helper cell type 2 ratio (6). In ischaemic stroke patients an impaired nocturnal MLT excretion has been associated with impaired cell-mediated immunity and changes of lymphocyte subsets (7). Relevant to RA, melatonin can promote collagen-induced arthritis in mice (8) and stimulate primary cultures of synovial macrophages from RA patients to produce interleukin-12 as well as nitric oxide (9).

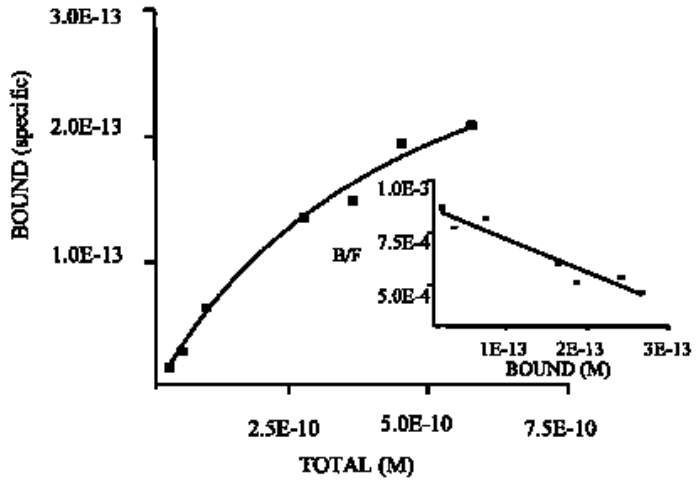

Fig. 2. Saturation isotherm and Scatchard plot of ^{125}I-melatonin in intact synovial macrophages from RA patients. Synovial macrophages were purified by adherence of $3x10^6$ synovial fluid cells/ml in RPMI 1640, 10% foetal calf serum for 2 h at 37° C, 5% CO_2. Triplicates samples of 1 ml of RPMI 1640, 1% foetal calf serum containing 2.5×10^5 viable synovial macrophages were incubated in presence of ^{125}I-melatonin at the reported concentrations with or without an excess concentration of melatonin (3×10^{-7} M) for 1 h at room temperature. The curve and the Scatchard plot are from a representative experiment out of 3 performed. Non-linear regression analysis gave the Scatchard plot (inset) which describes one binding site with Kd:$4.79^{-10} \pm 0.64^{-10}$ M and Bmax:$3.81^{-13} \pm 0.38^{-13}$ M which corresponds to 151 ± 15 fm/10^5 cells.

Here we compared the circadian rhythm of melatonin in serum from RA patients with that of healthy controls. We also measured the melatonin content in synovial fluid from RA patients and whether synovial macrophages express melatonin-specific binding sites.

All patients and healthy controls were informed about the aim of this study and gave their consent. The patients, hospitalised for active RA, were 6 females and 4 males, mean age 57.8 ± 13.2 while the controls were 4 females and 2 males, mean age 50.1 ± 18.2. The concentration of melatonin was significantly higher in RA sera at 8.00 p.m., 0.00 p.m. and 8.00 a.m. with a level of significance of $p < 0.02$, $p < 0.05$ and $p < 0.02$ respectively. In addition, a sort of plateau was observed in RA patients regarding melatonin levels. The melatonin content of synovial fluid from four RA patients was found of 79.8 ± 38.9 pg/ml. Synovial macrophages from RA patients showed a specific binding site for melatonin with a Kd of $4.79^{-10} \pm 6.4^{-11}$ M and a B_{max} of 151 ± 15 fm/10^5 cells (Fig. 1).

These results indicate that melatonin production in RA patients seems to be higher than in healthy controls at the beginning of the night and in early morning. All patients were hospitalised for active RA and showed the typical circadian variation of symptoms with joint stiffness and pain being more prominent in the early morning. Interestingly, the plasma concentration of melatonin was higher in the patients in the early morning too. Melatonin production in humans shows an inverse relationship with age (3). In our case the healthy controls were younger than the RA patients, therefore the modest although significant difference in serum melatonin concentration may well be associated to the underlying pathological state. Most interesting, melatonin was found to be present at a rather high concentration in synovial fluid from RA patients. In general, melatonin in body fluids is believed to originate from the blood. Here, it is noteworthy that the synovial fluid was collected in the late morning when the serum melatonin concentration is possibly much lower both in RA patients and controls (Fig. 1). This might indicate that synovial melatonin is produced *in situ.* It has been, in fact, recently reported that human immunocompetent cells may synthesise melatonin (10). To our knowledge, this is the first report showing the presence of melatonin in the synovial fluid. Whether melatonin is present at similar concentration in synovial fluid from healthy individuals or patients with other joint diseases will be matter of further studies. Whatever the origin, melatonin can bind specific binding sites in activated synovial macrophages with a binding affinity consistent with both its serum and synovial fluid concentration. Due to its peculiar ability to enhance pro-inflammatory cytokine production (4, 9), melatonin might thus play a pathogenic role in RA and possibly drive the circadian rhythm of the RA clinical symptoms (i.e. morning stiffness and gelling). Inhibitors of melatonin synthesis or melatonin antagonists might therefore be of therapeutic value in RA patients.

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