

The effect of individualized diet challenges consisting of allergenic foods on TNF- α and IL-1 β levels in patients with rheumatoid arthritis

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Objective. To investigate the effect of individualized diet challenges consisting of allergenic foods, defined by the skin prick test (SPT), on tumour necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) levels in patients with rheumatoid arthritis (RA).

Methods. Twenty patients with a positive SPT response for food extracts and 20 with a negative SPT response were enrolled. None of the patients had active disease. All patients were fasted for the most common allergenic foods for 12 days and then allocated to two groups according to SPT results. Food challenges were performed with allergenic foods in the prick-positive group (PPG) and with corn and rice in the prick-negative group (PNG) for a period of 12 days. Then, allergenic foods were excluded from the PPG patients' diet and corn and rice were removed from the PNG patients' diet. Clinical examinations were performed after fasting (baseline), at the end of the challenge phase and at the end of the re-elimination phase. Stiffness, pain, tender and swollen joint counts, health assessment questionnaire (HAQ), Ritchie's articular index, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and serum TNF- α and IL-1 β levels were measured.

Results. TNF- α ($P < 0.01$), IL-1 β ($P < 0.05$), ESR ($P < 0.05$) and CRP ($P = 0.001$) levels and all of the clinical variables, except HAQ, were increased with food challenges in the PPG. These increases were also recorded after the re-elimination phase. In the PNG, no significant change was seen in any of the variables, except pain ($P < 0.05$). During the study, important differences were observed for most of the variables between the two groups. Thirteen (72%) patients in the PPG and three (18%) in the PNG experienced disease exacerbation with challenges. This aggravation continued after elimination.

Conclusions. Our results suggest that individualized dietary revisions may regulate TNF- α and IL-1 β levels in selected patients with RA.

KEY WORDS: Rheumatoid arthritis, Cytokines, Diet therapy.

Food allergy is a complex immune mechanism that occurs in many parts of the body with diverse results [1]. It is extremely important to identify people who have food allergy because of the association of allergy with chronic disorders. The diagnosis of food allergy is based upon a careful history, physical examination, specific tests for immunoglobulin E (IgE), such as the skin prick test (SPT), the radioallergosorbent test and oral food challenges [1]. Food allergies can be determined with the SPT, which is an easy and cheap method. However, a food elimination/challenge trial is a good and reliable way to confirm a food allergy suggested by the SPT [2].

Food allergy was recorded in 1953 as a causative factor in some cases of arthritis [3]. Recent studies have reported that many of the signs and symptoms of rheumatoid arthritis (RA) may be linked to food allergies [4]. In many studies, several allergen-free or hypoallergenic dietary manipulations have been shown to ameliorate clinical manifestations of RA [5, 6], but the mechanisms by which this occurs are unknown.

On the other hand, recent research has uncovered the important role of cytokines which promote inflammation, such as tumour necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). Also, TNF- α and IL-1 are considered to be the key cytokines in the development of RA [7]. Evidence supports the correlation of levels of disease activity in RA and the progression of joint damage with levels of TNF- α and IL-1 [8]. In RA, TNF- α appears to be

primarily responsible for driving inflammation, while IL-1 plays a critical role in destruction of cartilage and bone. IL-1 exists in two forms, IL-1 α and IL-1 β , which are transcribed from closely related but distinct genes. IL-1 α is primarily bound to the cell membrane while IL-1 β is the predominant extracellular form of IL-1.

In reviewing the literature, it becomes clear that there are few studies investigating the mechanisms of the relationship between diet and TNF- α and IL-1 β . It was reported that fish oil can suppress proinflammatory cytokine production in RA patients [9]. Several studies have shown that n-3 fatty acid supplementation can reduce the production of IL-1 and TNF- α , and results in an important reduction in morning stiffness duration and tender joint count in patients with RA [10, 11].

However, the relationship between food allergy and proinflammatory cytokines in RA is still unknown. Therefore, we investigated the effect of individualized diet challenges consisting of allergenic foods on TNF- α and IL-1 β levels in patients with RA.

Patients and methods

Patients

This study was performed on patients with RA, according to the criteria of the American College of Rheumatology [12], in our

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out-patient clinic. The SPT with food extracts was applied to these patients for most allergenic foods. Twenty RA patients with a positive SPT response for at least one food and 20 patients with a negative SPT response were enrolled. All of the patients had a disease duration of at least 2 yr and had functional status class I–II as defined by the American College of Rheumatology [13]. None of the patients had clinically active disease, which is defined by the presence of at least two of the following criteria: (i) morning stiffness duration >30 min; (ii) six or more tender joints; (iii) three or more swollen joints; and (iv) erythrocyte sedimentation rate (ESR) >28 mm/h. All of the patients continued their previous disease-modifying anti-rheumatic drug medication at the start of the study. Patients who had a history of dietary manipulation for any reason (e.g. weight loss) were not included. On the other hand, intake of non-steroidal anti-inflammatory drugs (NSAIDs), antihistamines or glucocorticoids affects SPT results. Also, cytokine levels may be different in patients with and without NSAID therapy, since they may be effected by NSAIDs. Therefore, patients who had a history of taking NSAIDs, antihistamines or glucocorticoids for at least a month before the SPT were not included. However, paracetamol intake was not restricted during the study, except on clinical assessment days.

The study was approved by the local ethics committee. All of the participants were hospitalized to ensure dietary compliance and to prevent disease activation due to environmental effects. All patients were informed about the study protocol but not which dietary regimen they received. A consent form was obtained from all patients.

One patient was excluded from the study because of a positive SPT for rye flour. One patient with a negative SPT response was withdrawn since disease aggravation occurred due to severe urinary infection. Three patients (one with a positive SPT and two with a negative SPT response) were excluded at the elimination phase owing to low diet compliance. Thirty-five patients completed the study (18 with a positive SPT response, 17 with a negative SPT response).

The SPT

The SPT was performed for 31 kinds of food, using extracts (Stallergenes, France). No tests of red meat or milk or dairy products were performed since these extracts were not imported due to the risk of bovine spongiform encephalopathy. The SPT was performed on the volar side of the forearm with commercially available food extracts. The patient's skin was wiped with ethanol and allowed to dry. The inner aspect of the forearm was marked with a skin-marking pen. A sterile prick lancet was used to make a small prick through the drop and a new lancet was used for each allergen. A drop of each test extract was placed on the skin using a dropper. Histamine and saline solutions were used for positive and negative controls. The diameters of resulting wheal and flare reactions were recorded after 15 min. A positive SPT response was defined as a wheal at least 3 mm larger than the negative control wheal.

Dietary manipulations

Elimination phase. A low-allergen diet from which the most common allergenic foods had been eliminated was given to all RA patients for 12 days. During the elimination phase, dairy products, eggs, meat, fish, refined sugar, wheat, wheat flour, corn, rice, nuts, citrus fruits, tomato and coffee were avoided. The menus were based around cooked vegetables, legumes and fruit compote. However, freshly baked bread, which is eaten in every meal in Turkish culture, is a staple food. Therefore, rye bread without

wheat flour, which is known to be less allergenic than wheat in RA (34 and 54% respectively) [14], was given in limited daily amounts during the dietary manipulations.

Challenge phase. After the elimination phase, RA patients were allocated into two groups according to the SPT results. Eighteen patients who had a positive response were designated as the prick-positive group (PPG). For each patient, prick-positive foods were added in increased amounts to the patient's diet in the PPG. Therefore, the dietary regimen was different for each subject in the challenge phase. The patient ate all of the prick-positive foods every day. This individualized diet was continued for 12 days.

Seventeen patients had a negative response for all of the allergen food extracts in the SPT. The two foods were added in increased amounts to patient's diet in the prick negative group (PNG) to determine the placebo response to dietary challenge. Corn, which is known to be the most allergenic food in RA patients in percentage terms (57%), and rice, which is not defined as an allergenic food, were selected for this reason [14]. This diet, consisting of corn and rice, was given for 12 days.

Re-elimination phase. After the food challenges, prick-positive foods for each patient were removed from the patient's diet in the PPG. In the PNG, corn and rice were excluded from the daily diet. The re-elimination phase was continued for 12 days.

Clinical and laboratory follow-up

Clinical and laboratory assessments of the subjects were recorded after the elimination phase (baseline), at the end of challenge phase and re-elimination phase. Clinical evaluations and dietary manipulations were made by different researchers. Also, the examiner who performed the clinical examination was blinded to the patient groups.

Morning stiffness duration was evaluated in terms of minutes, based on the patient's report. Each patient's arthralgia at rest was determined with a visual analogue scale (VAS) with pain severity degrees from 0 to 10 cm. The numbers of tender and swollen joints were determined separately. The health assessment questionnaire (HAQ) [15] was used to evaluate functional disability and the Ritchie articular index (RAI) [16] to assess joints.

For the laboratory assessment, the ESR (Westergren method) and serum C-reactive protein (CRP), TNF- α and IL-1 β levels were measured. A nephelometric method was used for measuring CRP levels (Beckman Array Protein System, USA). Serum TNF- α and IL-1 β levels were determined with enzyme-linked immunosorbent assays using commercial kits (KHC3011 and KHC0011 respectively; BioSource, USA). The intra-assay and inter-assay coefficients of variation were 4.4 and 7.5% respectively for TNF- α and 4.4 and 6.7% for IL-1 β .

Statistical analysis

The statistical analysis was performed with SPSS 10.0 for Windows. All of the data were examined for normality. Normally distributed data were expressed as the mean and s.d. or as the median and range. Comparison of groups with normally distributed values was performed using the unpaired samples *t*-test, otherwise the Mann–Whitney *U* test was used. The paired-samples *t*-test and Wilcoxon rank test were used to compare normally and non-normally distributed data within groups, respectively. Values with *P* < 0.05 were accepted as statistically significant.

TABLE 1. Demographic features of the patients in the two groups

	Prick-positive group	Prick-negative group	<i>P</i>
Age (yr): mean (s.d.)	46.1 $\pm$ 8.7	48.4 $\pm$ 10.2	NS
Men/women	2/16	1/16	NS
BMI (kg/m ²): median (range)	28.5 (21.2–46.4)	29.7 (23.7–46.4)	NS
Disease duration (months): median (range)	54 (24–240)	48 (24–240)	NS

NS, not significant.

TABLE 2. Comparison of clinical and laboratory data at the end of the challenge and re-elimination phases with baseline in both groups [median (range)]

	Prick-positive group			Prick-negative group		
	Baseline	Challenge phase	Re-elimination phase	Baseline	Challenge phase	Re-elimination phase
Stiffness (min)	15 (1–30)	32.5 (10–60) [#]	52.5 (10–120) [#]	15 (1–30)	10 (1–45)	10 (1–60)
Pain (VAS)	3 (1–7)	5 (1–10) [‡]	6.5 (1–10) [‡]	4 (2–6)	4 (1–6) [†]	4 (2–8)
Tender joints	1 (0–5)	2 (0–6) [‡]	2.5 (0–6) [‡]	1 (0–5)	1 (0–3)	1 (0–4)
Swollen joints	1 (0–2)	1.5 (0–14) [†]	1.5 (0–15) [†]	1 (0–2)	0 (0–3)	0 (0–5)
RAI score	11.5 (5–30)	15.5 (2–57) [†]	16.5 (4–66) [†]	10 (0–33)	8 (1–32)	9 (0–34)
HAQ score	3 (0–13)	4 (0–14)	4 (0–18)	3 (0–15)	4 (0–15)	5 (0–17)
ESR (mm/h)*	20.3 $\pm$ 7.9	28 $\pm$ 15.6 [†]	31.2 $\pm$ 14.8 [‡]	18.2 $\pm$ 6.4	21 $\pm$ 6.8	23.4 $\pm$ 12.1
CRP (mg/dl)	0.4 (0.3–0.5)	1 (0.3–5.4) [#]	1.2 (0.3–7.6) [#]	0.5 (0.3–0.5)	0.4 (0.3–1.6)	0.4 (0.3–3.8)

*Mean $\pm$ s.d.; all other data are median (range).

[†]*P* < 0.05; [‡]*P* < 0.01; [#]*P* = 0.001 compared with baseline values.

Results

The patients' demographic properties are shown in Table 1 and their clinical and laboratory values in Table 2. At the start of the trial (baseline), no differences in demographic features, clinical and laboratory values were observed between the groups (*P* > 0.05).

ESR and CRP levels and all clinical variables except HAQ increased with food challenges in the PPG. These increases were also recorded after re-elimination. In the PNG, no significant difference was found in any of variables, except pain (*P* < 0.05), during the study. However, pain decreased with food challenges (*P* < 0.05) (Table 2).

Important differences were found between the two groups in pain, stiffness duration, the numbers of tender and swollen joints and CRP levels, but not in ESR levels and HAQ scores. Also, the RAI was found to differ between the two groups at the end of re-elimination phase (*P* < 0.05) (Table 3).

TNF- α (*P* < 0.01) and IL-1 β levels (*P* < 0.05) increased in the PPG but not in the PNG during the study. These levels were significantly different between the two groups at the end of the challenge and re-elimination phases (*P* < 0.05) (Figs 1 and 2).

Important disease aggravation, in terms of morning stiffness duration, pain, the numbers of tender and swollen joints, ESR and CRP levels, occurred in 13 patients (72%) in the PPG with food challenges. These exacerbations continued during the 12 days of observation in the re-elimination phase in 12 patients. Disease aggravation disappeared in only one patient in the re-elimination phase. However, disease activity increased in three patients (18%) in the PNG with food challenges and continued during the re-elimination phase. No difference was observed in body mass index (BMI) between the two groups during the study. No patients in either group showed significant weight loss at the end of trial.

Discussion

In oral food challenge trials, the suspected allergenic food is removed from the diet for a period of 7–15 days [1, 17]. The challenge period follows the elimination diet. In the present study,

TABLE 3. Comparison of significance levels between two groups in the challenge and re-elimination phases

	<i>P</i> value	
	Challenge phase	Re-elimination phase
Stiffness (min)	0.001	0.001
Pain (VAS)	<0.05	<0.05
Tender joints	<0.05	<0.05
Swollen joints	<0.05	<0.05
RAI score	NS	<0.05
HAQ score	NS	NS
ESR (mm/h)	NS	NS
CRP (mg/dl)	<0.001	<0.01

NS, not significant.

we fasted the patients for 12 days and then performed food challenges with all of the allergenic foods defined by the SPT. We observed statistically significant differences in clinical variables, except RAI and HAQ, with the challenges in the PPG compared with the PNG. However, the changes in serum CRP, TNF- α and IL-1 β levels differed significantly between the two diet groups, but not in ESR. The most commonly measured laboratory markers of disease activity in RA are the ESR and CRP. In recent studies, it was reported that CRP is more sensitive than ESR as a marker of disease activity because ESR is additionally affected by several factors, such as age, sex, anaemia, elevated fibrinogen and immunoglobulin levels, renal failure, pregnancy, and abnormal red blood cell morphology [18].

On the other hand, 13 of 18 patients in the PPG experienced disease exacerbation after intake of allergenic foods. Interestingly, this flare did not settle during the 12 days of observation in the re-elimination phase in 12 of 13 patients. Thus, it appears that food allergy may be a triggering factor rather than a causative factor in the aetiology of RA. The interaction of foreign food proteins with recognition components of the immune system may result in the production of inflammatory mediators that then recruit and activate effector cells. The interaction of antigens with

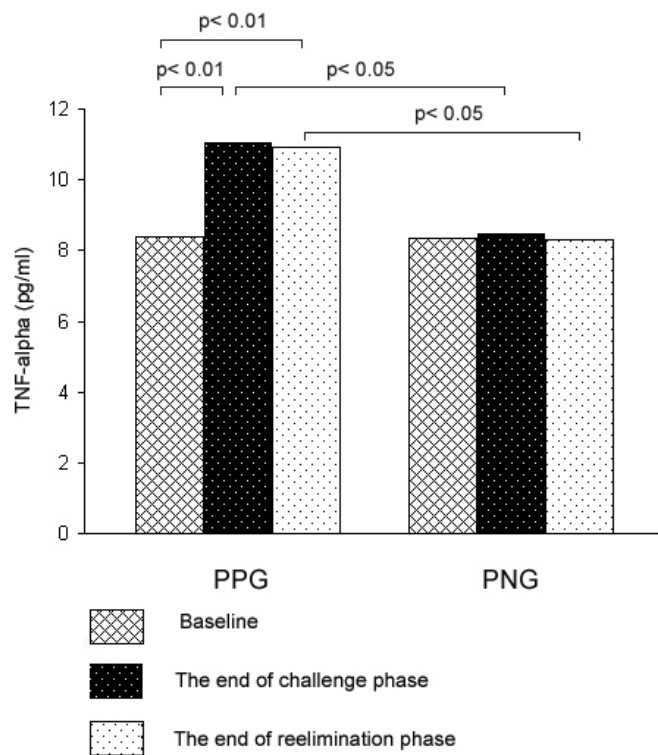


FIG. 1. TNF- α levels in the prick-positive group (PPG) and the prick-negative group (PNG) during the study.

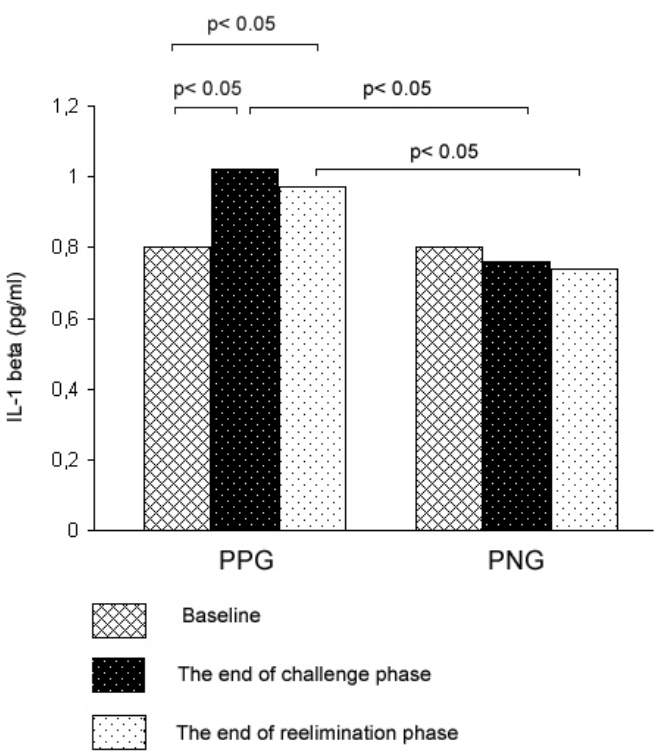


FIG. 2. IL-1 β levels in the prick-positive group (PPG) and the prick-negative group (PNG) during the study.

macrophages may subsequently identify the immunogenicity of the antigen or its ability to induce tolerance [19]. Also, it is well known that TNF- α and IL-1 β are mainly produced by activated macrophages in the inflammation cascade [20]. In the food allergy process, food antigens may activate macrophages and cause the release of TNF- α and IL-1 β from these cells. Also, Borish *et al.* [21] have shown the IgE-dependent production of TNF- α and IL-1 β by human circulating blood monocytes. Allergen-induced IgE may also stimulate the secretion of these cytokines by mononuclear phagocytes and may contribute to the inflammation cascade of allergic responses. However, the increase in proinflammatory cytokine expression may trigger the pathological immune process of RA. On the other hand, in atopic subjects, allergen-induced IgE antibodies and food antigens bind to mast cells (MCs). This leads to activation of MCs and to release of inflammatory mediators such as TNF- α and IL-1 β . However, in recent histological studies, localized accumulations of MCs have been demonstrated in the synovial tissue and at cartilage erosion sites in RA [22, 23]. Also, increased activation of MCs has been found in these locations in rheumatoid specimens and synovial cell cultures [23]. MC activation may contribute to the inflammatory processes in the rheumatoid lesion.

On the other hand, it is not known exactly why food allergy and dietary manipulations affect some RA patients and not others. It is possible that an exogenous agent, such as a food antigen, can initiate a pathological immune process in a genetically susceptible individual. Studies in families show that first-degree relatives of RA patients develop the disease at a higher rate than the general population [24]. Also, a family history of allergies seems to be an important factor predisposing a person to develop allergic disease [25]. However, many studies have reported that inherited predisposition to RA is associated with the DRB1 genes encoding human leucocyte antigen DR4 (HLA-DR4) and HLA-DR1 molecules [26, 27]. Interestingly, it was found that HLA-DR4 and HLA-DR7 alleles are significantly associated with predisposition to atopy [28]. Howell *et al.* [29] showed a relationship

between HLA-DRB1 and peanut allergy. Also, some studies reported that HLA-DR genotypes and HLA-DRB1 alleles modulate serum IgE levels and the IgE response to common major allergens [30, 31]. However, the mechanism of the association between the expression of particular HLA genes and the predisposition to RA and allergy is unclear. It is possible that the intake of a certain food in an increased amount may trigger the pathological immune process of RA in people who have the HLA genes that play an important role in susceptibility to RA and allergic diathesis.

After the intake of allergenic foods, the increases in TNF- α and IL-1 β levels may be important not only in the aetiopathogenesis of RA but also in treatment approaches. The inhibition of TNF- α and IL-1 appears to be a novel complementary strategy for the treatment of RA at present [32]. In this study, we demonstrated that TNF- α and IL-1 β levels are affected by diet challenges performed with allergenic foods. In this condition, the exclusion of certain foods from the patient's diet may regulate the production of TNF- α and IL-1 β . Individualized diet therapy may decrease the requirement for anti-TNF- α antibodies and recombinant human IL-1 receptor antagonist (IL-1ra). However, a much larger study would be needed to confirm that individualized diet manipulations affect the TNF- α and IL-1 β levels in patients with RA.

Rheumatology	Key messages
	• Food allergy may be a triggering factor rather than a causative factor in RA aetiology. • TNF-α and IL-1β levels may be affected by dietary challenges performed with allergenic foods.

The authors have declared no conflicts of interest.

References

1. Sicherer SH. Manifestations of food allergy: evaluation and management. *Am Fam Physician* 1999;59:415–24.
2. Sicherer SH. Food allergy: when and how to perform oral food challenges. *Pediatr Allergy Immunol* 1999;10:226–34.
3. Kaufman W. Food-induced, allergic musculoskeletal syndromes. *Ann Allergy* 1953;11:179–84.
4. Gaby AR. The role of hidden food allergy/intolerance in chronic disease. *Altern Med Rev* 1998;3:90–100.
5. Darlington LG, Ramsey NW, Mansfield JR. Placebo-controlled, blind study of dietary manipulation therapy in rheumatoid arthritis. *Lancet* 1986;1:236–8.
6. van de Laar MA, van der Korst JK. Food intolerance in rheumatoid arthritis. A double blind, controlled trial of the clinical effects of elimination of milk allergens and azo dyes. *Ann Rheum Dis* 1992;51:298–302.
7. Taylor PC. Anti-cytokines and cytokines in the treatment of rheumatoid arthritis. *Curr Pharm Des* 2003;9:1095–106.
8. Dayer JM. Interleukin 1 or tumor necrosis factor-alpha: which is the real target in rheumatoid arthritis? *J Rheumatol Suppl* 2002;65:10–5.
9. Grimble RF, Howell WM, O'Reilly G *et al.* The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. *Am J Clin Nutr* 2002;76:454–9.
10. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. *Br J Nutr* 2001;85:251–69.
11. Endres S, Ghorbani R, Kelley VE *et al.* The effects of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989;320:265–71.
12. Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
13. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *JAMA* 1949;140:659–62.
14. Darlington LG, Ramsey NW. Review of dietary therapy for rheumatoid arthritis. *Br J Rheumatol* 1993;32:507–14.
15. Fries JF, Spitz P, Kraines RG. Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;23:137–45.
16. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakas TG, Grievson P. Clinical studies with an articular index for the assessments of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968;147:768–9.
17. Skoldstam L, Magnusson KE. Fasting, intestinal permeability, and rheumatoid arthritis. *Rheum Dis Clin North Am* 1991;17:363–71.
18. Richardson C, Emery P. Laboratory markers of disease activity. *J Rheumatol Suppl* 1996;44:23–30.
19. Pike MC, Synderman R. Structure and function of monocytes and macrophages. In: McCarty DJ, Kopman WJ, eds. *Arthritis and allied conditions*. London, 1993:347–76.
20. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. *Arthritis Rheum* 1995;38:151–60.
21. Borish L, Mascali JJ, Rosenwasser LJ. IgE-dependent cytokine production by human peripheral blood mononuclear phagocytes. *J Immunol* 1991;146:63–7.
22. Tetlow LC, Woolley DE. Mast cells, cytokines, and metalloproteinases at the rheumatoid lesion: dual immunolocalisation studies. *Ann Rheum Dis* 1995;54:896–903.
23. Woolley DE, Tetlow LC. Mast cell activation and its relation to proinflammatory cytokine production in the rheumatoid lesion. *Arthritis Res* 2000;2:65–74.
24. Emery P, Buch M. The aetiology and pathogenesis of rheumatoid arthritis. *Hosp Pharmacist* 2002;9:5–9.
25. Reich K, Westphal G, Konig IR *et al.* Cytokine gene polymorphisms in atopic dermatitis. *Br J Dermatol* 2003;148:1237–41.
26. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 2002;4(Suppl. 3):S265–72.
27. Fugger L, Svejgaard A. Association of MHC and rheumatoid arthritis: HLA-DR4 and rheumatoid arthritis—studies in mice and men. *Arthritis Res* 2000;2:208–11.
28. Aron Y, Desmazes-Dufeu N, Matran R *et al.* Evidence of a strong, positive association between atopy and the HLA classII alleles DR4 and DR7. *Clin Exp Allergy* 1996;26:821–8.
29. Howell WM, Turner SJ, Hourihane JO, Dean TP, Warner JO. HLA class II DRB1, DQB1 and DPB1 genotypic associations with peanut allergy: evidence from a family-based and case-control study. *Clin Exp Allergy* 1998;28:156–62.
30. Mansur AH, Williams GA, Bishop DT *et al.* Evidence for a role of HLA DRB1 alleles in the control of IgE levels, strengthened by interacting TCR A/D marker alleles. *Clin Exp Allergy* 2000;30:1371–8.
31. Young RP, Dekker JW, Wordsworth BP *et al.* HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994;24:431–9.
32. Zwerina J, Hayer S, Tohidast-Akrad M *et al.* Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. *Arthritis Rheum* 2004;50:277–90.