



Correlation between cytokine concentrations in bronchial secretions and PaO₂/FiO₂ ratio

by insertion of a sterile suction catheter into the endotracheal tube, advanced until it was felt to wedge. Sterile saline, 0.5 mL/kg was instilled and immediately aspirated into a sputum trap. Samples were centrifuged to remove cells and we analysed the resulting supernatants for TNF α and IL-8 concentrations with EIA (R&D Quantikine, Abingdon Ltd.). We assessed with Spearman correlation the relation between concentrations of proinflammatory cytokines, duration of aortic cross-clamping, CPB time, and the arterial to inspired oxygen ratio (PaO₂/FiO₂) 2 h after CPB was stopped.

11 of the 15 patients had postoperative PaO₂/FiO₂ ratios less than 300 mm Hg. In three patients this ratio was less than 200 mm Hg, which constitutes one of the diagnostic criteria for ARDS. None of these patients had a cardiogenic cause for decreased oxygenation (pulmonary capillary wedge pressure less than 18 mm Hg). Low ratios did not correlate with long cross-clamp or CPB times.

Plasma cytokine concentrations rose after CPB but did not correlate with the PaO₂/FiO₂ ratio. By contrast, we found that raised bronchial concentrations of TNF α 10 mm after cross-clamp release predicted poor postoperative oxygenation ($p=0.0001$). Bronchial TNF α was not detected in patients whose postoperative PaO₂/FiO₂ ratios were greater than 300 mm Hg, whereas the highest concentration of TNF α (59 pg/mL) occurred in a patient who subsequently developed a PaO₂/FiO₂ ratio below 200 mm Hg. TNF α concentrations did not relate to the duration of aortic cross-clamping. We could not use IL-8, a chemokine commonly implicated in the pathogenesis of postoperative pulmonary dysfunction, to predict poor postoperative oxygenation since the correlation between IL-8 and PaO₂/FiO₂ ratios 10 min after release of the aortic cross-clamping just failed to reach significance ($p < 0.06$).

We show that pulmonary dysfunction after routine

cardiac surgery may be predicted by a slight rise in TNF α concentrations in bronchial secretions. This novel finding was perhaps facilitated by the small diluting volume from which we obtained bronchial aspirate. Conventional bronchoalveolar lavage with 2 mL/kg of saline can potentially mask small changes in cytokine production. Also of note was the lack of correlation between TNF α concentrations and potential triggers, such as the duration of aortic cross-clamping or CPB. The TNF α response to a given challenge varies widely in health³ and disease,⁴ prompting the suggestion that TNF α production is genetically determined.

- 1 Uter PM, Suter S, Girardin E, et al. High bronchoalveolar levels of tumour necrosis factor and its inhibitors, interleukin-1, interferon, and elastase in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992; **145**: 1016–22.
- 2 Donnelly SC, Strieter RM, Girardin E, et al. Interleukin-8 and the development of adult respiratory distress syndrome in at-risk patient groups. *Lancet* 1993; **341**: 643–47.
- 3 Westendorp RGJ, Langermans JAM, Huizinga TWJ, et al. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997; **349**: 170–73.
- 4 Zipp F, Weber F, Huber S, et al. Genetic control of multiple sclerosis: increased production of lymphotoxin and tumour necrosis factor- α by HLA DR2 + T cells. *Ann Neurol* 1995; **38**: 723–30.

Department of Clinical Anaesthesia, Royal Group of Hospitals, Belfast BT12 6BJ, UK; Department of Immunobiology, Queen's University, Belfast; and 210-2057 West 3rd Avenue, Vancouver, BC, V6J 1L4, Canada (H E Gilliland)

Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility

Joyce Eskdale, Janet McNicholl, Paul Wordsworth, Beth Jonas, Tom Huizinga, Max Field, Grant Gallagher

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting about 1% of the population. Hereditary factors are important in susceptibility to RA; HLA-DR associations probably account for only a third of the overall genetic contribution. Cytokine concentrations are raised in RA, with high interleukin-10 titres present in serum and synovial fluid.¹ Interleukin-10 is a major immunoregulatory cytokine, usually considered to mediate potent downregulation of the inflammatory response. Some effects of interleukin-10, however, are not anti-inflammatory; for example, activation of B cells to promote autoantibody production.² The human interleukin-10 gene is highly polymorphic, with point mutations and two microsatellite loci, IL10.G and IL10.R, located 1.2 kb and 4.0 kb upstream of the coding region, respectively.³ We have shown previously that haplotypes containing particular combinations of the IL10.G and IL10.R alleles can be associated with differential interleukin-10 secretion.⁴

We investigated whether there were RA-associated differences in allele frequency at these two microsatellites. We enrolled 103 white patients from Glasgow University Centre for Rheumatic Disease and 148 from Nuffield Orthopaedic Centre, Oxford. These patients were compared with 94 and 87 white controls from Glasgow and Oxford, respectively. We also enrolled 61 African-American patients from the Grady African-American Rheumatoid Arthritis cohort at the Rheumatology Clinic, Grady Memorial Hospital, Atlanta, USA, and 38 African-American controls. Patients fulfilled the 1987 American

IL10R alleles	Controls	Patients	p*	Odds ratio (95% CI)
Glasgow				
R1	0	0
R2	106 (56.4)	143 (69.4)	0.007	1.7 (1.14-2.64)
R3	76 (40.4)	60 (29.1)	0.018	0.61 (0.39-0.92)
R4	6 (3.2)	3 (1.5)
R5	0	0
Oxford				
R1	0	1 (0.3)
R2	106 (60.9)	208 (70.3)	0.037	1.5 (0.97-2.13)
R3	63 (36.2)	73 (24.7)	0.008	0.58 (0.38-0.87)
R4	5 (2.9)	13 (4.4)
R5	0	1 (0.3)
Atlanta				
R1	3 (4.0)	1 (0.8)
R2	55 (72.3)	106 (86.9)	0.018	2.5 (1.22-5.23)
R3	18 (23.7)	13 (10.7)	0.014	0.38 (0.18-0.84)
R4	0	2 (1.6)
R5	0	0

*p values for individual alleles are by χ^2 test.

Comparison of IL10.R alleles in RA patients from different locations

College of Rheumatology criteria for RA. We used our previously published methods to analyse microsatellite alleles, assess interleukin-10 production, and carry out statistical analysis.^{3,4} All patients and controls were allelotyped in one laboratory (JE). Allele calls were assigned independently by two investigators.

We found no association between RA and the IL10.G microsatellite in any group (not shown). We compared RA and control groups by means of the Monte Carlo simulation at the allelotyped IL10.R locus. The overall distribution of IL10.R alleles varied between patients and controls in all three groups (Glasgow, $p=0.014$; Oxford, $p=0.023$; Atlanta, $p=0.009$) and this was due to a single allele (table). In all three groups the IL10.R2 allele was significantly over-represented at the specific expense of the IL10.R3 allele, in the RA patients. Genotypes containing the IL10.R3 allele are associated with significantly lower concentrations of IL-10 secretion when compared with IL10.R3 negative genotypes (median, 1850 vs 2456 pg/mL; $p=0.011$).⁴

Thus, the raised concentrations of interleukin-10 secretions found in RA may have a direct genetic basis, since the allele associated with low interleukin-10 was under-represented in these three groups of patients.

Our data show that the IL10.R microsatellite is associated with RA in people of different origins, which is important to our understanding of the aetiology of the disease. Interleukin-10 (and its gene) could be involved in many aspects of RA pathogenesis. High concentrations of interleukin-10, although inefficient at neutralising the proinflammatory cascade, may actively contribute to disease progression by elevating autoimmune activity in RA^{1,2} and other conditions.⁵ Although the actions mediated by interleukin-10 are diverse, our observations suggest that the interleukin-10 gene, through promotion of high interleukin-10 concentrations, may have an active role in the aetiology and pathogenesis of RA.

- Cash JJ, Splawski JB, Thomas R, et al. Elevated interleukin-10 levels in patients with rheumatoid arthritis. *Arthritis Rheum* 1995; **38**: 96-104.
- Perez L, Orte J, Brieva JA. Terminal differentiation of spontaneous rheumatoid factor-secreting B-cells from rheumatoid arthritis patients depends on endogenous interleukin-10. *Arthritis Rheum* 1995; **38**: 1771-76.
- Eskdale J, Kube D, Tesch H, Gallagher G. Mapping the human IL-10 gene and further characterisation of the 5' flanking sequence. *Immunogenetics* 1997; **46**: 120-28.
- Eskdale J, Gallagher G, Verweij CR, Keijsers V, Westerdrop RGJ, Huijzinga TWJ. IL-10 secretions, in relation to the haplotypic structure of the human IL-10 locus. *Proc Natl Acad Sci USA* 1998; **95**: 9465-70.

- de la Vega JR, Vilplana JC, Bior A, Hammond L, Bottazzo GF, Mirakian R. IL-10 expression in thyroid glands: protective or harmful role against thyroid autoimmunity. *Clin Exp Immunol* 1998; **113**: 126-35.

Department of Surgery (J Eskdale; e-mail ggva26@udcf.gla.ac.uk) and Centre for Rheumatic Diseases, University of Glasgow, Queen Elizabeth Building, Glasgow Royal Infirmary, Glasgow G31 2ER, UK; Immunology Branch, DASTLR, ACID, Centers for Disease Control and Prevention, Atlanta, GA, USA; Division of Rheumatology, Department of Medicine, Emory University, Atlanta; Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; and Department of Rheumatology, University Hospital Leiden, Leiden, Netherlands

Saliva test as ovulation predictor

Didi D M Braat, Jesper M J Smeenk, Arentje P Manger, Chris M G Thomas, Sebastiaan Veersema, Johannes M W M Merkus

There is an increasing demand for cheap self-tests to predict the fertile period. Although the urine luteinising-hormone test seems to be an effective predictor of ovulation, cheaper small microscopes have been introduced to predict fertile days by detecting microscopically ferning in saliva drops. Ferning has been suggested to correlate with 17β -oestradiol serum or saliva concentrations and, therefore, with the fertile period. The mini-microscope has been proposed as a reliable method for natural family planning, as well as for prediction of the fertile period.² Although there have been some negative responses,³ these tests are now widely available and are recommended by infertility specialists and patients' groups. We investigated prospectively the reliability of these tests.

We asked 36 women with regular menstrual cycles to participate in the study. In 30 women the day of ovulation could be confirmed: group 1 ($n=17$) by ultrasound and serum luteinising-hormone measurements; group 2 ($n=13$) by a shift in the basal body-temperature chart. Every morning a drop of saliva was dried and assessed microscopically with a mini-microscope in group 1 (by JS) and a normal light microscope in group 2 (by AM). The fertile period was defined as from 5 days before until 1 day after ovulation.⁴ Tests were positive with the appearance of ferning or intermediate (some) ferning, as proposed by the manufacturer. If no ferning could be seen the test was negative. The sensitivity was 53% (group 1) and 86% (group 2); the specificity was 72% (group 1) and 14% (group 2). The likelihood ratio for a positive test was 1.9 in group 1 and 1.0 in group 2, and for a negative test was 0.7 in group 1 and 1.0 in group 2.

We used the same test for ten postmenopausal women and in ten men (performed by AM). In eight of the ten postmenopausal women and in all of the men the test was positive.

We compared 17β -oestradiol concentrations for 31 saliva samples (11 women in group 1) with serum 17β -oestradiol values. There was a strong correlation between saliva and serum ($p<0.0001$), but no correlation could be detected between the 17β -oestradiol concentrations in saliva and the ferning aspect. These findings strongly suggest that the saliva ferning test is unreliable for predicting the fertile period and its use should, therefore, be discouraged.

- Barbato M, Pandolfi A, Guida M. A new diagnostic aid for natural family planning. *Adv Contracept* 1993; **9**: 335-40.
- Rotta L, Matechova E, Cerny M, Pelak Z. Determination of the fertile period during the menstrual cycle in women by monitoring changes in crystallization of saliva with the PC2000 IMPCON minimicroscope. *Ceska Gynekol* 1992; **57**: 340-52.
- Berardono B, Melani D, Ranaldi F, Giachetti E, Vanni P. Is the salivary "ferning" a reliable index of the fertile period? *Acta Eur Fert* 1993; **24**: 61-65.