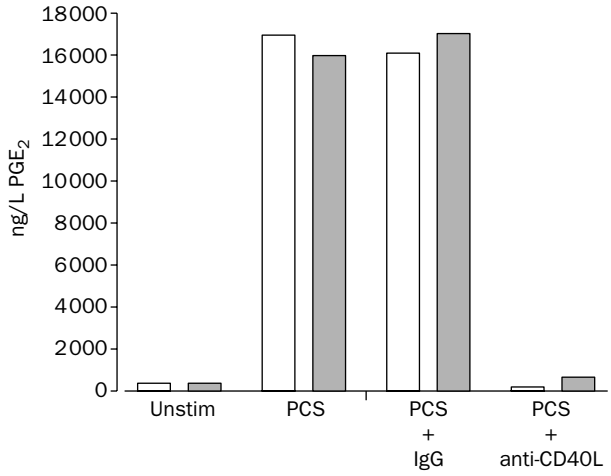

Figure 2: **PGE2 production in two strains of human lung fibroblasts by CD40 ligand present in platelet concentrate supernatants.**
Unstim=medium alone; PCS=1/20 dilution platelet concentrate supernatants; anti-CD40L=CD40 ligand antibody.

negative control antibody. The CD40 ligand antibody completely abrogated the induction of PGE_2. At 1/200 dilution, platelet concentrate supernatants still induced PGE_2 production.

High concentrations of bioactive CD40 ligand in platelet transfusions, perhaps in concert with other mediators,[2] might explain the persistence of transfusion reactions even when white blood cells are removed before platelet storage. The intensity of the febrile response may depend upon the level of CD40 ligand in the platelet preparation, the sensitivity of the patients to CD40 ligand, and whether the patients are taking steroids or non-steroidal anti-inflammatory drugs that would inhibit the production or activity of cyclo-oxygenase and thereby prostaglandin synthesis. If platelet soluble CD40 ligand is confirmed as the cause of these febrile responses, then we speculate that keeping the release of CD40 ligand to a minimum, or removing free CD40 ligand before transfusion, may effectively reduce adverse events after platelet transfusions. Moreover, individuals whose platelets are especially rich in CD40 ligand might prove less suitable as donors for transfusions.

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Department of Microbiology and Immunology (R P Phipps PhD, J Kaufmann MS), **and Department Pathology and Laboratory Medicine** (N Blumberg MD), **University of Rochester Cancer Center, Rochester, NY 14642, USA**

Correspondence to: Dr Richard Phipps
(email: Richard_Phipps@urmc.rochester.edu)

Association between sciatica and *Propionibacterium acnes*

Alistair Stirling, Tony Worthington, Mohammed Rafiq, Peter A Lambert, Tom S J Elliott

We hypothesised that the inflammation seen around the nerve root in patients with sciatica may be caused by microbial infection. We used a newly developed serological test to diagnose deep-seated infections caused by low virulent gram-positive microorganisms. 43 of 140 (31%) patients with sciatica tested positive. Intervertebral disc material from a further 36 patients with severe sciatica who had undergone microdiscectomy was cultured for the presence of microorganisms. 19 of these patients (53%) had positive cultures after long-term incubation. *Propionibacterium acnes* was isolated from 16 of the 19 (84%) positive samples. Low virulent microorganisms, in particular *P acnes*, might be causing a chronic low-grade infection in the lower intervertebral discs of patients with severe sciatica. These microorganisms could have gained access to the spinal disc after previous minor trauma.

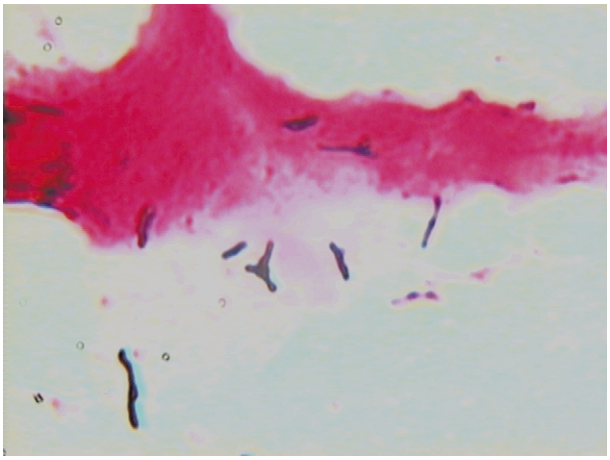
Although low back pain and sciatica are some of the most common reasons for consultation in primary care,[1] their cause is poorly understood. Marshall and colleagues[2] have shown raised serum immunoglobulin concentrations in patients with sciatica. Discogenic radiculitis, which is caused by pressure, is also associated with an inflammatory process, and cytokines, including interleukin-1α, have been linked to lumbar disc herniation. Surgically induced disc lesions in animals have also resulted in an inflammatory reaction in the nucleus pulposus, with the production of IgG in surrounding tissues. Inflammation around the nerve root may be due to a local autoimmune reaction.

The inflammation associated with sciatica could also have a microbial cause, possibly low virulent microorganisms, as seen in prosthetic hip infections. We used an ELISA based on an exocellular antigen produced by gram-positive bacteria (lipid S) for the diagnosis of deep-seated infections caused by low virulent pathogens, including coagulase-negative staphylococci and propionibacteria. The ELISA had previously facilitated diagnoses of prosthetic hip[3] and central venous catheter infections,[4] endocarditis, and pyogenic spondylodiscitis.

We used this serological test to investigate 140 patients with sciatica presenting to a specialist clinic. These patients were originally selected as controls for a study on spondylodiscitis, however, 43 of 140 (31%) had raised serum IgG titres to the lipid S antigen. No patient had reported any infection in the past 6 months.

A further 36 patients with severe sciatica were clinically assessed, and had lumbar magnetic resonance imaging. Discogenic radiculitis was subsequently diagnosed and the patients had microdiscectomies to relieve the pain. Disc tissue (about 2×2×5 mm) removed during operation was examined for presence of microorganisms. Stringent aseptic precautions were taken to minimise the risk of contamination in clean wound culture.

All samples were cultured in Robertson's cooked meat enrichment broth, incubated at 37°C and subcultured at 2, 7, and 21 days onto blood agar plates containing 7% defibrinated horse blood (Oxoid Ltd, Basingstoke, UK). Subcultures were incubated at 37°C under anaerobic conditions for 7 days, and inspected for microbial growth. Tissue samples that were large enough (n=11) were also cultured directly by impression onto 7% blood agar plates, and embedded into nutrient agar (Oxoid



Light photomicrograph of microdiscectomy tissue showing *Proprionibacterium acnes* (×1000)

Ltd, Basingstoke, UK). We also prepared thin sections, which were gram-stained and examined for presence of microorganisms.

Tissue from 19 of 36 patients (53%) gave positive cultures in the enrichment technique within 7 days of incubation. *Proprionibacterium acnes*, coagulase-negative staphylococci, and *Corynebacterium propinquum* were isolated from 16 (84%), two (11%), and one (5%) of 19 positive cultures, respectively. Blood samples were obtained for lipid S antibody estimation from 15 of 19 patients with positive cultures. Seven (47%) had positive serological tests. 16 of the 17 (94%) patients with negative cultures had negative tests to the lipid S antigen. More patients with positive cultures had positive serological tests than did those with negative cultures (36% [7/19] *vs* 5% [1/17], p=0·0438; Fisher's exact test). C-reactive protein was measured in 25 of the 36 (69%) patients. Two of these 25 (8%) had C-reactive protein concentrations above 10 mg/L, and *P acnes* was isolated in both.

Discetomy tissue was concurrently examined from 14 controls presenting with other spinal disorders, including scoliosis (n=3), trauma (3), myeloma (2), and degenerative disc disease (6). None of these samples yielded positive cultures after long incubation. There was a significant difference between the number of patients with sciatica who had positive tissue cultures (53% [19/36]) as compared with controls (0% [0/14] p=0·0003). Additionally, 12 of 14 (86%) controls had negative serum antilipid S IgG titres.

P acnes was seen in six of 11 (55%) tissue samples that were cultured directly onto blood agar. Between 10 and 100 colony forming units were isolated from each sample. Gram-stained smears of the tissue samples embedded in agarose also showed gram-positive branching rods after incubation (average size 3·0×0·3 μm) (figure). Microorganisms were not detected in any of the 11 gram-stained thin sections examined by direct microscopy, suggesting that they are present only in low numbers.

In this preliminary study we have detected raised concentrations of a specific serum antibody to a glycerophospholipid (lipid S), an exocellular bacterial cell-wall component. We also recorded a corresponding presence of low virulent microorganisms, predominantly propionibacteria, in a high proportion of patients with sciatica.

We postulate that patients with sciatica sustain a breach in the mechanical integrity of a spinal disc, possibly from minor trauma, which allows access by low virulent microorganisms, thereby initiating or stimulating a chronic inflammatory response with accompanying symptoms. This hypothesis is consistent with the anatomical distribution of disc degeneration, with greater stresses on the distal regions of the spine. The observation that disc protrusions detected by magnetic resonance imaging are frequently symptomless, perhaps becoming symptomatic only after infection, also lends support to this hypothesis.

Previous epidural injections could be a further source of microorganisms. However, 53% (10/19) from whom bacteria were isolated had not had a pre-operative epidural injection, and 41% (7/17) of the tissue culture negative patients had. These findings suggest that epidural injections were not the source of the microorganisms.

Over the past two decades the skin commensals coagulase-negative staphylococci and propionibacteria, mainly thought of as contaminants when isolated from patients, are increasingly being recognised as agents of infection. Tunney and colleagues[5] have shown the presence of *P acnes* and coagulase-negative staphylococci in situ on prosthetic joints studied at revision arthroplasty. Our results also suggest that these microorganisms, particularly *P acnes*, might be associated with chronic low-grade infection in the lower intervertebral discs of patients with severe sciatica.

The need to incubate disc material from patients for up to 7 days to isolate the propionibacteria might be why these microorganisms have not been previously associated with sciatica. The long generation time of propionibacteria makes these microorganisms ideal candidates for chronic infection. *P acnes* produces many exocellular virulence factors (including lipase, proteinase, hyaluronidase, neuramidase, and phospholipase C), which may contribute to its pathogenicity. These microorganisms may cause prosthetic hip infections, but also might be associated with the inflammation seen in sciatica, and may possibly even be a primary cause of this disorder.

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Royal Orthopaedic Hospital, Northfield, Birmingham, UK (A Stirling FRCS, M Rafiq FRCS); **Department of Clinical Microbiology, University Hospital, Edgbaston, Birmingham, B15 2TH, UK** (T Worthington PhD, Prof T S J Elliott FRCPath); **Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle, Birmingham, UK** (P A Lambert DSc)

Correspondence to: Prof T S J Elliott
(e-mail: tom.elliott@university-b.wmids.nhs.uk)