Case Report

Common Aspects of Human and Primate Seronegative Arthritis

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ABSTRACT. A 27-year-old female lowland gorilla developed an asymmetric oligoarthritis 3 months post-partum. There was no evidence of an antecedent gastrointestinal or genitourinary infection. Serum was negative for rheumatoid factor and antinuclear antibody. Synovial fluid revealed 2000 white blood cells with negative cultures and polarized microscopy. Studies on synoviocytes were the following: (1) FACS analysis revealed surface expression of a B27-like epitope of the cells. (2) Analysis of intracellular clearance kinetics of arthritogenic organisms showed peak intracellular colony-forming units at 48 hours after bacterial invasion, and clearance by 13 days post-invasion. (3) Interferon-γ (0.1–10.0 ng/ml) accelerated intracellular microbicidal pathways in a dose-dependent fashion. These findings closely parallel those seen in human synoviocytes of patients with spondyloarthropathy. Primate and human seronegative arthritis share clinical and immunologic features, as well as aspects of host:pathogen defense mechanisms. The interplay of genetic and microbial factors underlying this arthritis appears to be conserved across these species boundaries. (J Rheumatol 2004;31:2300–4)

Key Indexing Terms:
ARTHRITIS GORILLA BACTERIA PRIMATE

While murine models of rheumatic diseases have been instructive in some instances, such as in systemic lupus erythematosus, spontaneous arthritis in the animal kingdom has received less attention in the rheumatology literature. We describe our recent experience with a gorilla mimicking human spondyloarthropathy (SpA).

Reactive arthritis (ReA), a subtype of SpA, refers to a nonseptic inflammatory joint disease accompanying an infection[1]. The pathogenesis of ReA is unresolved, but studies of synovial tissues in ReA following *Yersinia*, *Salmonella*, and *Chlamydia* all point to local persistence of microbial antigens in the joint as a key component in maintaining the chronic synovitis. Since there is evidence of persistence of such antigens for prolonged periods after the initial infection[2,3], the concept has arisen that defective clearance of the arthritogenic pathogen may play an important role in the pathogenesis of ReA. There has been some evidence that HLA-B27 may modulate intracellular clearance of *Salmonella*[4], but few studies have addressed the question using primary synoviocytes as host target cells. It has also been proposed that impairment in nitric oxide production may account for the altered kinetics of intracellular clearance[5], although the HLA allele specificity of this event remains unresolved[6]. We utilized the opportunity to study host:pathogen interactions using the synoviocytes from our case of primate seronegative arthritis. Although we had no access to control synoviocytes to address the specificity of any MHC allele affect, we were able to observe that the kinetics of intracellular clearance of an arthritogenic pathogen paralleled exactly that seen in the human counterpart[7].

CASE REPORT

The Toronto Zoo in Toronto, Ontario, recently requested a consultation from our rheumatology service. Josephine, a 27-year-old female lowland gorilla (*Gorilla gorilla*) had recently developed arthritis. She presented with a one-week history of anorexia and lameness, particularly favoring her left hand and foot. She had had no previous medical problems and there was no observed trauma. There was no history of diarrhea or urinary tract infection. She was 3 months post-partum from a stillbirth, thought secondary to placental insufficiency. No cohabitating gorilla in the zoo had past or current symptoms to suggest a rheumatological problem.

Physical examination under general anesthesia revealed normal vital signs. Her weight was 100 kg. General physical examination by the veterinary staff was normal. There were no skin lesions. Joint examination revealed that the second and third metacarpophalangeal (MTP) joints on the left hand were warm and effused. Range of motion was preserved. The right hand was normal. There was swelling of the left first MTP joint with an extensor deformity of the first interphalangeal joint on the same toe. No other musculoskeletal abnormalities were noted.

Her white blood cell count was 6.1 × 10^9/l, hemoglobin 116 g/l, and

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platelets 265 × 10^9/l. Serum creatinine was 85 µmol/l and uric acid 160 µmol/l. Serum was negative for rheumatoid factor and antinuclear antibody. Synovial fluid from an aspirate of the second left metacarpophalangeal joint revealed 2000 leukocytes, with negative culture and crystal analysis by polarized microscopy. Radiographs of the hand and feet showed no evidence of erosive disease. Radiographs of the sacroiliac joints were not obtained.

Josephine was treated with naproxen sodium 375 mg bid for a presumed seronegative arthritis. She responded well with resolution of her effusions and resumption of normal activity. The drug was stopped after 2 months, with no recurrence of her arthritis at 6 months' followup.

Synoviocyte harvesting and FACS analysis. Synovial fluid was obtained using sterile technique from Josephine's affected joints. The fluid was added to α-modified Eagle's medium with 20% fetal calf serum (FCS). A primate adherent fibroblast cell line was subsequently obtained from cells that grew out of the fluid and maintained by splitting once weekly. Control synoviocytes were obtained from a B27/human $ß_2$-microglobulin double transgenic rat derived from a breeding pair generously provided by Dr. J. Taurog (Dallas, Texas, USA) and normal Lewis rats. They were grown out from synovial tissue explants as described[8]. These rodent fibroblasts were similar in appearance to Josephine's fibroblasts. FITC-conjugated mouse anti-human HLA-B27 antibody (clone m3) was obtained from Serotec (Oxford, England). A monolayer of the primate synoviocytes was removed from the flask with cell dissociation buffer (Gibco). Cell numbers were adjusted to 10^6/ml, and 1.0 ml portions of cells were stained with the monoclonal antibody (1:5) at 4°C for 1 h. The stained cells were analyzed in a Becton-Dickinson FACScan.

Clearance of arthritogenic bacteria. Quantitative invasion assays were performed as described[9]. Briefly, bacteria were grown to mid-log phase, washed in phosphate buffered saline (PBS), and resuspended in Dulbecco's modified Eagle's medium (Gibco) with 5% FCS. Then 0.1 ml aliquots were added to the PBS-washed cell monolayer containing 0.3 ml media. To make immediate contact between cells and bacteria, the plate was spun for 10 min at 1800 rpm. The bacteria were incubated with the synoviocytes for 2 h at 37°C, at a bacteria:cell ratio of 2:1 to 10:1. After washing, gentamicin 100 µg/ml was added for 1 h to kill the extracellular bacteria. Cells were harvested at different time points and colony-forming units (CFU) were determined after cell lysis (with 0.2% Triton X-100) to calculate the total intracellular bacterial load. The organisms used were arthritogenic strains of *Salmonella typhimurium* and *Yersinia enterocolitica 0:3* recovered, respectively, from patients with ReA during an epidemic of *Salmonellosis* and from a patient with post-*Yersinia* ReA. Interferon-γ (IFN-γ; Endogen, Cambridge, MA, USA) was used to observe its effect on clearance of bacteria. For comparison, synoviocytes harvested from a B27+ patient with SpA were studied in a similar manner.

Measurement of nitric oxide (NO) production. NO production of the synoviocytes was measured as nitrate in the supernatants of the infected cells in the presence or absence of IFN-γ. The accumulation of nitrate was determined with the diazotization reaction with the Griess reagent (0.1% naphtylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid). Cell-free supernatants of the infected cells with or without IFN-γ were collected at days 0, 1, 2, 3, 7, 10, and 13. The samples (100 µl) were mixed with equal volumes of Griess reagent and incubated at room temperature for 10–30 min. Absorbance was measured at 570 nm and nitrite concentrations were interpolated from standard curves prepared with $NaNO_2$ [5-7].

Results. To address surface expression of HLA-B27 or cross-reactive determinants, the synoviocytes were stained with a monoclonal HLA-B27 antibody. Synoviocytes were stained at a level intermediate between the positive control (synoviocytes from a B27-transgenic rat) and the negative control (synoviocytes from a matched nontransgenic Lewis rat; Figure 1).

To analyze bacterial clearance by synoviocytes, the cells from Josephine were incubated with 2 arthritogenic pathogens, *S. typhimurium* and *Y. enterocolitica*, at different timepoints (Figure 2A). For both pathogens, intracellular CFU rose over day 1 and 2 and declined steadily to day 13. This is comparable to an analysis of clearance kinetics using human HLA-B27+ synoviocytes derived from a patient with ReA (Figure 2B). Clearance kinetics of the pathogens of both primate and human target cells reflected comparable time courses[7].

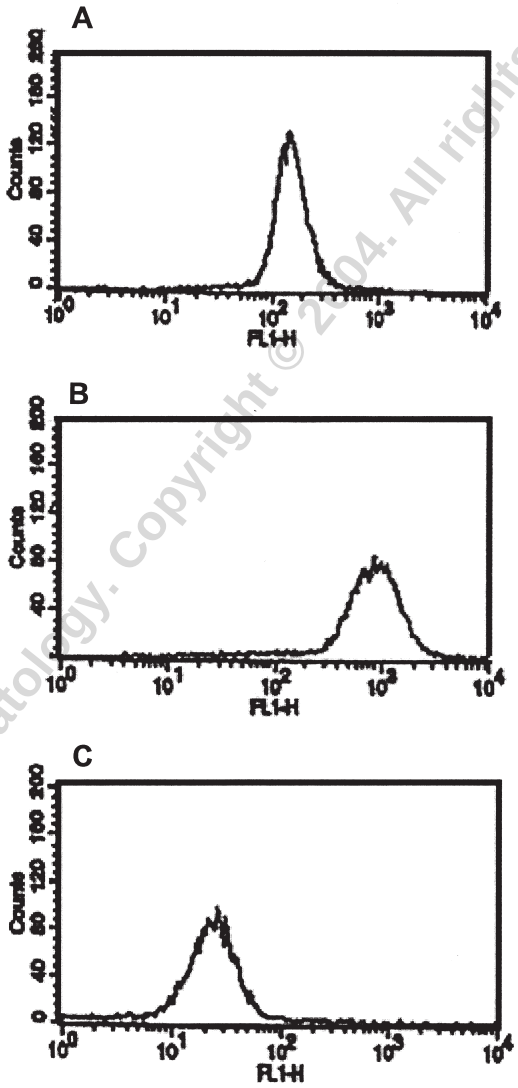

Figure 1. FACS analysis of synoviocytes using a murine antibody of synoviocytes isolated from the primate spondyloarthropathy case (A), synoviocytes from a B27-transgenic rat (B), and synoviocytes from a non-transgenic Lewis rat (C).

Because of the implicated microbicidal effects of IFN-γ and NO, we addressed the production of NO by the primate synoviocytes cocultivated with *S. typhimurium* for 7 and 10 days, respectively. Figure 3A indicates that at these 2 timepoints, increasing amounts of IFN-γ had no consistent effect on NO production. This is in contrast to the dose-dependent microbicidal activity of IFN-γ represented in Figures 3B and 3C. IFN-γ thus appears to accelerate clearance of *Salmonella*, but not via NO pathways.

DISCUSSION

There is no perfect animal model for seronegative or seropositive arthritis. Case reports suggest primates are afflicted with several joint diseases comparable to humans. There are reports of chronic osteoarthritis in lowland gorillas[10].

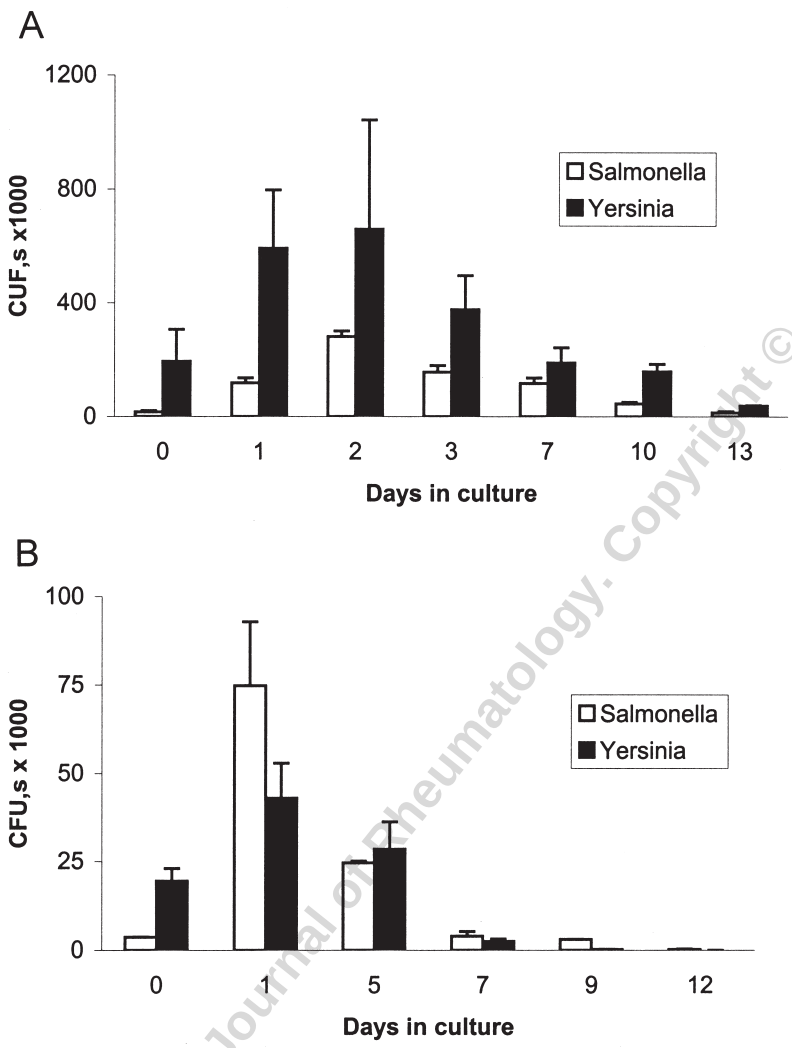

Figure 2. Kinetics of intracellular clearance of *S. typhimurium and Y. enterocolitica* in primate synoviocytes. After synovial cells of gorilla were cocultured with *Salmonella* and *Yersinia* at a 1:2 to 1:10 cell/bacterium ratio for 2 h at 37°C, the cells were overlaid with fresh incubation medium containing 10 µg gentamicin/ml and incubated 60 min at 37°C. After washing with PBS, synoviocyte cells were lysed at different timepoints. Analysis showed peak intracellular colony forming units (CFU) at 48 h after bacterial invasion, and clearance by 13 days post-invasion (A). Intracellular clearance of *Salmonella* and *Yersinia* from a B27 positive patient with SpA is shown (B).

Rheumatoid-like disease has been described in rhesus monkeys (*Macaca mulatta*) and a lion-tailed macaque (*Macaca silenus*)[11-13]. There has also been evidence of calcium pyrophosphate dihydrate-like disease in these same monkeys and Barbary ape[14].

In our case there were features mimicking human SpA, including a seronegative asymmetric oligoarthritis occurring after a potential infectious event (stillbirth). In this case, however, it may be more accurate to define this simply as seronegative arthritis since there was no documented antecedent infection or skin or gastrointestinal disease. Radiographs of the sacroiliac joints was not obtained. Most research in the area of primate arthropathies has focused on the occurrence of SpA-like disease. SpA has been documented as a pan-mammalian phenomenon, with high penetrance in many groups. As many as 25% of bears[15] and 35% of rhinoceroses[16] have been reported to manifest this clinical problem. The diagnosis of SpA was clearly identified as a population phenomenon in gorillas in 1989[17]. While it is quite reasonable to consider the possibility of selective disadvantage in the wild (for lame or slower-moving animals), that question likely pertains to zoo specimens as well. The frequency of 20% in skeletal samples of lowland gorillas is indistinguishable from that subsequently identified in zoologic parks[18,19]. This contrasts with a SpA

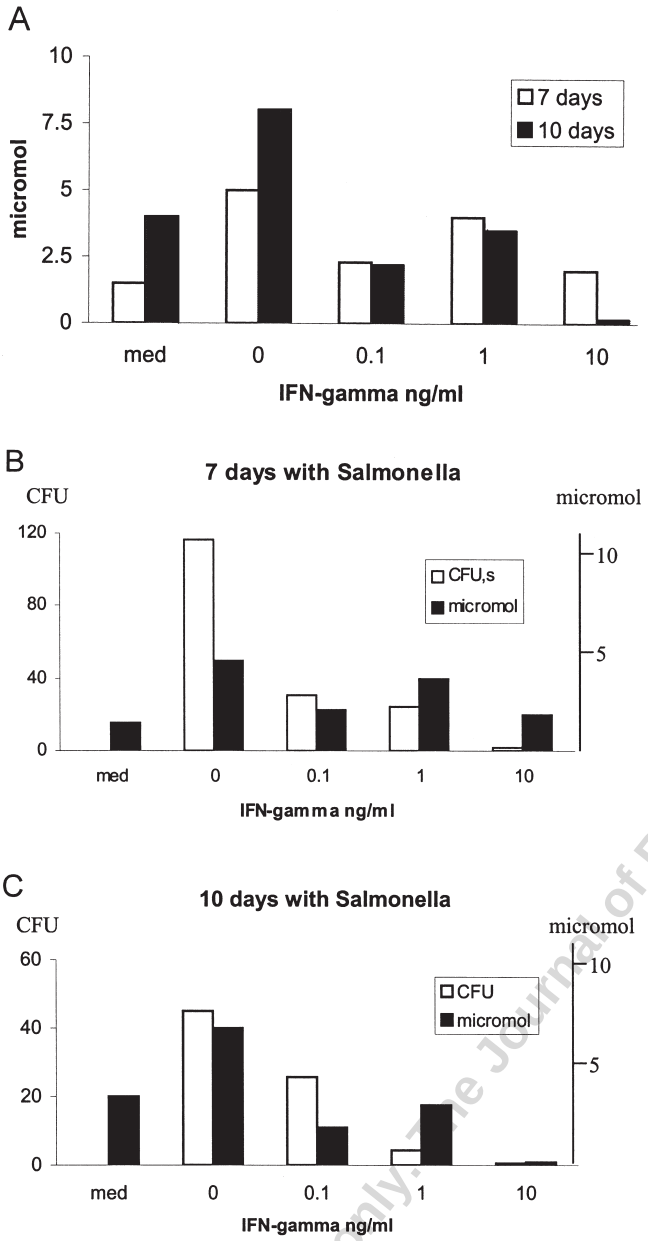

Figure 3. (A) NO production by primate synoviocytes inoculated with *S. typhimurium*. Interferon-γ (IFN-γ) does not increase NO production. IFN-γ does accelerate intracellular clearance of *Salmonella* at 7 days (B) and 10 days (C) in a dose-dependent manner, but not via the NO pathway.

prevalence of 1% to 2% in humans. This study of gorillas described an asymmetrical subchondral erosive disease with perilesional, smooth, billowy new bone formation in the peripheral joints, and erosions, ankylosis, and syndesmophytes in the sacroiliac joints and spine. These findings are in keeping with the pattern of ankylosing spondylitis (AS) in humans. Resistance to nonsteroidal antiinflammatory drug (NSAID) therapy has proved to be common in gorilla SpA and sulfasalazine (SSZ) is now considered standard treatment for this disease[19]. In our case, Josephine responded well to NSAID, and SSZ therapy was not instituted.

There has recently been interest in the MHC classifications among primates. Studies of MHC Class I genes have shown that homologs for HLA-A, B, E, and F are preserved in primates. Chimpanzees have shown preserved B locus alleles homologous to HLA-B15, HLA-B48, HLA-B57/58, and HLA B27/7[20]. Similar homology has been reported between the rhesus macaque Mamu-B*03 molecule and human HLA-B27[21,22]. A Gogo-B (*Gorilla gorilla*) allele (B*01) with limited nucleotide sequence similarity to HLA-B27 was reported in association with gorilla SpA[19,23]. Subsequently, a National Zoo Committee project documented that a B27 epitope is extremely common in gorillas. In our study, the use of antisera for detection of HLA-B27 is complex, with issues of specificity still unresolved, especially in the gorilla. Using FACS analysis of the gorilla synovial fibroblasts we found evidence of an HLA-B27-like epitope on the surface of the cells.

The significance of this homology and association with clinical disease, however, remains unresolved in our case. Of the 3 gorillas described with the HLA-B27-like determinants, one developed a ReA after infection with *Shigella flexneri*[23] and one demonstrated clinical signs indistinguishable from AS[24]. An unrelated gorilla in the same zoo as one of the animals in this report also tested HLA-B27 positive by standard human leukocyte tissue-typing methods and displayed no clinical disease. A recent report analyzed the MHC class I cDNA expressed in a cohort of rhesus macaques who developed ReA after an outbreak of shigellosis[22]. The investigators identified molecules that could bind peptides similar to HLA-B39. However, they could not find any statistically significant relationship between any particular MHC Class I molecule and development of ReA. They did not identify a molecule similar to HLA-B27, suggesting perhaps that ReA could occur in their animal model in the absence of HLA-B27.

It would appear that the clinical and biological features in our primate recapitulate the human counterpart of seronegative arthritis. The synovial fibroblasts expressed a structure on the surface similar to HLA-B27. We recently published our experience with bacterial clearance in synoviocytes from SpA[7]. Challenge of the gorilla synoviocytes with intracellular pathogens showed clearance kinetics similar to human SpA synoviocytes[7]. NO is thought to be important in the primary response against arthritogenic bacteria, and IFN-γ has been known to be an efficient inducer of NO synthase in macrophages[25]. Our recent analysis on human synoviocytes confirmed an interaction of NO and IFN-γ in the clearance of intracellular pathogens, although the clearance profile was not clearly associated with B27 expression.

Our case illustrates that the cell biology of SpA may be common in diverse primate species, implying that the genetic susceptibility to this disease may be conserved across the species.

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