Aetiology of rheumatoid arthritis: an attempt to transmit an infective agent from patients with rheumatoid arthritis to baboons


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SUMMARY Thirty baboons were injected intravenously and intra-articularly with material from the joints of 19 patients with active rheumatoid arthritis or with control material. Fifteen of the 30 animals received synovial fluid cells or synovial membrane cells from 3 patients with seronegative arthritis. Ten animals received pooled cells from a total of 16 cases of seropositive arthritis. Five animals were given nonrheumatoid cells. No signs of arthritis were recognised in the 27 surviving animals during 3 years of observation. No significant biochemical, haematological, or serological changes occurred during this period, and no gross or microscopic evidence of synovial or systemic disease was found post mortem.

The theory that a bacterial or viral infection is aetiologically related to rheumatoid arthritis (RA) is tenaciously held despite many false leads and the absence of firm confirmatory evidence. A variety of experimental approaches in different disciplines has produced candidate organisms ranging from bacteria—in particular diphtheroids—to mycoplasmas and to viruses. There is no conclusive evidence linking diphtheroids or mycoplasmas to the disease. Similarly, a search for viruses by miscellaneous techniques has also been negative, though there is serological evidence of a relationship between Epstein-Barr virus infection and RA.

Another way to demonstrate an infective aetiology for RA is to attempt to transmit the disease to experimental animals. Warren and his colleagues claim to have transmitted RA to mice, chickens and rats by the injection of human synovial membrane extracts, but other workers have failed to reproduce these findings. Crocker et al. using complement-deficient mice, have found that the intraperitoneal injection of homogenates of RA synovial membrane produces inflammatory swelling of the tails and limbs of mice and occasional runting. Such changes do not necessarily indicate transmission of an infective agent, and as far as we can determine no confirmatory reports of this observation have appeared.

It is recognised that the host range of certain human viruses is restricted and that negative transmission experiments with common laboratory animals do not finally exclude the presence of a virus in human tissues. For example, human hepatitis B and non-A, non-B viruses grow in chimpanzees but not in small laboratory animals. It therefore appeared reasonable to attempt to transmit RA to nonhuman primates. This paper describes such an attempt by the inoculation of human synovial cells and cell preparations into the knee joints of baboons.

Materials and methods

Thirty female, immature and mature, 7.8–19.2 kg baboons were imported from Africa and held at Inveresk Research International, Edinburgh. They were housed in large gang cages and kept under close observation for 5 months. During this period they were examined clinically at regular intervals and underwent biochemical, haematological, serological, and radiological investigations. All the animals...
remained in good health during this period and none showed evidence of arthritis.

**Biochemical.** Before inoculation and at each examination after inoculation assays were made of: serum urea, creatinine, uric acid, aminotransferases, alkaline phosphatase, glucose, total protein, albumin, bilirubin, calcium phosphate, magnesium, and cholesterol.

**Haematological.** Estimates of haemoglobin, haematocrit, and erythrocyte sedimentation rates (ESR) were made at the same times as the biochemical assays.

**Serological.** Samples of blood were obtained 3 months before inoculation, immediately before inoculation and at 6, 9, 12, 17, and 23 months after inoculation. All sera were examined by a sensitised sheep cell agglutination test (SCAT) and with the RA latex reagent (Searle Diagnostic) for antiglobulin activity analogous to human rheumatoid factor (RF). In addition, polystyrene latex particles 0·81 μm diameter (Difco) were coated with IgG isolated from pooled baboon sera by fractionation on diethylaminoethyldiethylaminoethyl (DEAE) cellulose and used in parallel with the Searle reagent. In the latex systems inactivated sera were tested at dilutions of 1 in 10 and 1 in 20. Serial 2-fold dilutions of serum from 1 in 16 to 1 in 1024 were employed in the SCAT.

Specimens of sera were examined for antinuclear factor (ANF) by an indirect immunofluorescence technique employing alcohol-fixed, human blood films as substrate and for anti-DNA antiantibody by a modification of a haemagglutination technique. In the latter test pyruvic aldehyde-treated human blood group O cells are coated with highly polymerised DNA prepared from bovine thymus (BDH) and the sera titrated in Cooke microtitre plates at dilutions from 1 in 16 to 1 in 1024.

**Virological.** Sera were tested for antibody to influenza A and B, adenovirus, respiratory syncytial virus, *Mycoplasma pneumoniae*, mumps, herpes simplex, measles, parainfluenza, rubella, and hepatitis B surface antigen.

**Histopathological.** Comprehensive studies were made of the viscera; and of the synovial membranes, articular cartilage and periarticular structures of the left and right hip and knee joints, of the first (index) digital metacarpophalangeal and interphalangeal joints, and occasionally of other joints.

**PROCEDURES**

The 30 baboons were divided into 6 groups of 5 animals. Five groups were inoculated intravenously and into the right knee joint with material from 19 RA patients (Table 1). The remaining group was inoculated with control material.

**Group 1.** (Table 2). These animals received cells from a cultured synovial cell line that arose by cocultivation of Chang liver cells with RA synovial fibroblasts. The cells displayed cultural and morphological characteristics, and surface antigens, distinct from those of the original Chang cell cultures.

**Group 2.** (Table 2). These animals received cells from a synovial fluid effusion, washed and resuspended in cell culture medium. Cells from this fluid, established in culture, showed massive syncytial formation thought to indicate possible virus activity.

**Group 3.** (Table 2). These animals received cells cultured from RA synovial membrane that showed a high degree of DNA polymerase activity. The DNA polymerase estimations were undertaken because it had been suggested that viral reverse transcriptase might be present in RA synovial membranes. Investigations in our laboratories revealed that the polymerase was RNA-primed and DNA-dependent, of the type found in activated lymphocytes. It was not viral reverse transcriptase. Nevertheless the presence of this DNA polymerase appeared to be an indicator of an active disease process with lymphocyte stimulation.

**Group 4.** These animals were injected with Chang cells as a control for group 1.

**Group 5.** These animals received pooled.
sededented, and washed cells from specimens of synovial fluid from 7 seropositive RA patients.

*Group 6.* These animals received pooled dispersed trypsinised cells from 9 specimens of actively inflamed RA synovial membrane from 5 early and 4 late seropositive patients. This pooled preparation was more representative of the whole synovial membrane than the cells in culture which were fibroblastic in nature.

**Results**

**Clinical.** Frequent inspection of the animals during life did not reveal symptoms, signs, or radiological evidence of arthritis.

**Biochemical.** Occasional biochemical abnormalities were observed in individual animals but there was no evidence of significant variation between the groups. An anticipated decrease in alkaline phosphatase levels occurred in the serum of immature animals.24

**Haematological.** Haemoglobin values were constant throughout the study apart from occasional variations in individual animals. ESRs were increased only in animals developing local infection after injuries sustained during fights.

**Serological.** The SCAT remained uniformly negative throughout the study. The highest incidence of positive latex tests with the Searle reagent, including all animals in control group 4, was observed 23 months after injection (Table 3). The latex agglutination test that employed particles sensitised with baboon IgG did not change significantly at any time during the investigation. The test was negative in all animals before injection but weakly positive reactions (+) were recorded in one or more baboons in each group at various intervals. The test for ANF was positive in one animal in group 3 at this same period. This baboon also had a positive latex test on the same occasion. Haemagglutination titres for anti-DNA antibody ranged from 1/32 to 1/512, but rising antibody titres were not observed in any animal. A similar range of DNA-haemagglutination titres was found in a group of control baboons.

**Virological.** No significant differences in viral antibody titres were recorded with the exception of one animal in group 3, which developed a low rise in rubella antibody (8→32→64) 6 months after injection. This was not considered to represent a significant infection.

**Histopathological.** Three animals died during the study of causes unrelated to the experiment. At the close of the study the surviving animals were killed by preliminary sedation followed by intravenous pentobarbitone. The identity of test and control animals was not known to the prosectors. All animals were found to be in good physical condition. Body fat was evident in the larger mature survivors but was scanty by adult human standards. At necropsy no evidence was found in the inoculated baboons of active synovitis of the injected right knee, or of joints that had not been inoculated. Two examples of osteoarthritis (OA) of the femoral head were recognised; in one the disorder complicated a long-standing displacement or malformation of the femoral head associated with a healed fracture of the upper femoral shaft. One instance of OA of the knee was observed. A single interphalangeal joint was ankylosed, following trauma, while in several animals there was traumatic or infective amputation of distal phalanges or of finger nails. No signs were recognised in the viscera of infected animals of changes such as granuloma formation, serositis, or arteritis that would be anticipated in a proportion of cases of established human RA, and there was no evidence of significant systemic disease. Three animals displayed old pleural adhesions of limited extent. Lymphadenopathy was common in both right femoral and left axillary regions. There was a single instance of splenomegaly for which no explanation was found. Cardiac enlargement of unknown cause was shown in one animal. Atherosclerosis was not recognised. There was no evidence of pyelonephritis and none of hypertensive disease. No neoplasms were encountered.

Microscopic examination showed no evidence of inflammatory joint disease in the right knee synovium. Occasionally small deposits of haemosiderin crystals indicated sites of intra-articular bleeding. There were no synovial lymphocytic or plasma cell infiltrates, no synovial cell hyperplasia, no fibrin, and no foci of necrosis or of amyloid.

**Discussion**

The clinical and clinical laboratory definition of RA
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is by criteria such as those of the American Rheumatism Association.25 The pathological definition is debated; a series of anatomical and histological changes is characteristic but not pathognomonic.26-28 The significance of the present experiment must be judged by the extent to which these clinical, clinical laboratory, and morphological criteria have been reproduced. On this basis, the present attempt to transmit an infective agent from RA synovial tissue by means of cultured or cocultivated cells, or cells enzymically dispersed from synovial exudate, has clearly been negative: the animals showed no signs of articular abnormality and no gross or microscopic changes suggestive of RA developed. Serological markers of RA such as rheumatoid factor and ANF are regarded as criteria of lesser importance than the clinical signs of disease, particularly in the absence of joint changes. These markers were also essentially negative or, as in the case of the Searle latex tests for RF, became positive in all groups, a response that was presumed to be an aspect of aging unrelated to the nature of the inoculum. The serological tests for virus infection were undertaken as a background investigation, and the results have no significance, given the failure to transmit RA-like disease to the baboons. The agglutination of DNA-coated erythrocytes may have been a nonspecific effect of baboon sera.

The results of the present experiment provide no support for the hypothesis that RA may be caused by a transmissible, infective agent that persists in the synovial cells or synovial fluid of seropositive or seronegative cases. Several explanations may be advanced for this negative outcome.

Firstly, no transmissible agent may have remained in the injected cells. There has been no irrefutable evidence to date of the successful reproduction of an animal model of RA by the experimental injection or inoculation of material from human joints. However, there have been reports of the transmission by an agent from human synovial membrane injected into mice, chickens, and rats12-15 of vascular lesions of the feet, talipes deformity, or hip dislocation. Others16 provoked inflammatory swelling and tail and limb deformity in a proportion of C5-deficient mice injected intraperitoneally with homogenates of RA synovial membrane. No lesions were produced by the injection of synovial tissue from normal or OA joints.

Secondly, the baboons may not have been susceptible to a transmissible agent present in the inoculated synovial cells. The baboon is not known to be prone to natural polyarthritis, and, although deforming articular disease has occasionally been described in this species, the joint disorder is degenerative. No report of a natural inflammatory polyarthritis of the baboon has been found in the literature, though the sensitised baboon is capable of reacting to intra-articular antigenic challenge by the production of a synovitis not wholly dissimilar from RA in man.29

Whether RA occurs naturally in any animal species other than man remains debates. Brown et al.30 described an inflammatory arthritis in the gorilla that displayed some of the characteristics of human RA, and Schumacher et al.31 have reported an RA-like disease in dogs. Bywaters22-33 is among the few who have searched primate populations for evidence of chronic polyarthritis. In a radiological survey of 152 rhesus monkeys erosions and inflammatory changes were identified in the carpal and in several interphalangeal joints. Gillman and Gilbert34 found ankylosing osteophytes in one baboon of 50 that they examined; and Benditt and Eriksen35 described 3 rhesus monkeys with an RA-like inflammatory arthritis. There are reports of very small numbers of primates (rhesus monkeys) with RA-like disease.35-36

Thirdly, the incubation period of an infective agent transmitted to the baboon may be prolonged beyond the present period of observation. In this context there appear to be 2 main categories of slow virus infection.

The first category is due to conventional viruses with a nucleic acid and a protein coat or envelope. Pathological changes develop slowly. Nevertheless, the disease pattern is usually established within a 2-year period. In the present experiment the baboons were inoculated when immunologically competent, and this explanation for slow incubation does not seem acceptable.

The second category is one in which unconventional viruses such as those of the spongiform encephalopathies—scrapie, kuru, and Jacob-Creutzfeldt disease—take a very long time to manifest pathological changes. In these unconventional slow virus infections the pathological changes are characterised by a lack of inflammatory reaction. This is in pronounced contrast to the response of RA. If, therefore, a slow virus is implicated in the aetiology of RA it would be necessary to postulate an indirect effect, on immunoregulation for example rather than a direct effect as antigen. The second category of slow virus has no virus-coded antigen; indeed, the nature of the genetic information is obscure.

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