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RESEARCH COMMUNICATION

A COLUMN PURIFICATION PROCEDURE FOR THE REMOVAL OF LEUCOCYTES FROM PARASITE-INFECTED BOVINE BLOOD

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ABSTRACT

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A cellulose column procedure is described which removes white cells from bovine blood infected with *Babesia* and *Anaplasma*. The efficiency of this method was confirmed by the absence of white blood cell DNA in lysates from column-filtered infected as well as non-infected blood.

Recently there have been a number of reports on the construction of genomic libraries from a variety of organisms, including protozoan parasites (McCutchan, Hansen, Dame & Mullins, 1984; Cowman, Timmis & Kemp, 1984). Papers published to date describe protocols used to remove white blood cells from parasite-infected blood (Cowman *et al.*, 1984, Homewood & Neame, 1976). Lack of experimental detail frequently makes it impossible to establish the efficiency and reliability of the methods used. A basic requirement for the application of recombinant DNA techniques is the availability of DNA which is known to be free of contaminating DNA from other sources. In this paper we describe a method which results in a preparation which is free of detectable amounts of DNA from white blood cells and which can be used routinely for the isolation of DNA sufficiently pure to be used for the development of specific probes and the construction of genomic libraries.

Cross-bred, splenectomized oxen and heifers, born and reared at this institute under strict tick-free conditions for the purpose of live blood vaccine production, were used as infected and non-infected blood donors in this study. The *Babesia* strains used were needle-passaged, attentuated vaccine strains, namely, *Babesia bovis* S strain (De Vos, 1978) and the Australian *B. bigemina* strain G (Dalgliesh, Callow, Mellors & McGregor, 1981). The origin of *Anaplasma centrale*, also used in a live blood vaccine, was described by Potgieter (1979). Sterile infected blood was collected in 20 % ACD (13,4 g dextrose, 13,2 g sodium citrate, 4,8 g citric acid per litre) by canulating the jugular vein of an animal undergoing a primary reaction to the specific infection. Average parasitaemias (% of infected erythrocytes) were: *B. bovis*, 1,3 %., *B. bigemina*, 5,3 % and *A. centrale*, 33 %.

Whole blood was collected and centrifuged at 2000 rpm for 10 min. The supernatant and buffy coat were discarded. This process was repeated at least 4 times, or at least until the buffy coat was no longer evident. The erythrocytes were then divided into 2 equal aliquots and treated in 1 of 2 ways:

Method A: The red blood cells were lysed with an equal volume of lysis buffer (0,3 M sucrose, 1 % Triton X-100, 5 mM $MgCl_2$, 10 mM Tris HCl, pH 7,6). Pronase (20 mg $m\ell^{-1}$) was added and the mixture incubated for 2 h at 37 °C. The lysate was extracted twice with phenol, followed by 2 phenol/chloroform and 2 ether extractions. The aqueous phase was precipitated with ethanol, and the pellet was resuspended in TE buffer (10 mM Tris, pH 7,5, 1 mM EDTA).

Method B: A glass column (50 × 600 mm) was packed to a depth of 300 mm with dry Whatman CF-11 cellulose powder (Cat No. 11112). The red blood cell fraction was diluted with an equal volume of distilled water and passed through the column. No pressure was applied to the column and the suspension was allowed to filter through by gravity. To ensure complete lysis of all the parasites in suspension, the filtrate was lysed, extracted and precipitated as described in method A. DNA pellets were resuspended in TE buffer and aliquots were electrophorezed on agarose cells.

Large amounts of DNA were isolated from both non-infected and infected red blood cells purified by method A (Fig. 1D & 1C). A strong electrophoretic band was observed which ran in the same position on agarose gels as the DNA band from white blood cells (Fig. 1E).

DNA could be isolated by method B from all the infected samples tested (*B. bovis*, *B. bigemina* and *A. centrale*), but could not be detected in samples from uninfected blood (Fig. 1B). We found that only 5 % of the initial volume of red blood cells was retained by the column.

DNA from an uninfected animal was labelled by nick-translation (Southern, 1975) and hybridized to bovine

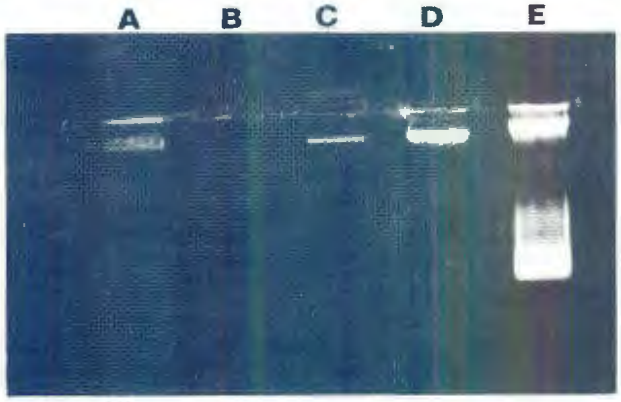

FIG. 1 Agarose gel electrophoresis of DNA isolated from *B. bovis*-infected blood
Lane A: *B. bovis* DNA from infected blood, method B
Lane B: DNA from uninfected blood, method B
Lane C: DNA from *B. bovis*-infected blood, method A
Lane D: DNA from red blood cell pellet, uninfected blood, method A
Lane E: White blood cell DNA
Electrophoresis was for 16 h at 40 V on a 1 % horizontal agarose gel in TA buffer (40 mM Tris-Acetate, 2 mM EDTA). DNA was visualized by staining with ethidium bromide (1 μg $m\ell^{-1}$) for 20 min. Gels were photographed on a UV transilluminator through a Kodak Wratten No. 25 filter

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DNA, *B. bovis*, *B. bigemina* and *A. centrale* DNA, extracted by methods A & B. No hybridization of the bovine probe was obtained with DNA extracted from *B. bigemina*, *A. centrale* and uninfected blood using method B (Fig. 2A, G and E). Some hybridization was obtained with the *B. bovis* DNA sample (Fig. 2D) indicating that it was not entirely free of white blood cell DNA.

FIG. 2 Autoradiogram of a Southern blot of 1 μg amounts of DNA isolated from infected and uninfected blood hybridized with 50 ng (10^6 dpm) of bovine leucocyte DNA.
Lane A: *B. bigemina*-infected blood, method B
Lane B: *B. bigemina*-infected blood, method A
Lane C: *B. bovis*-infected blood, method A
Lane D: *B. bovis*-infected blood, method B
Lane E: red blood cell extraction, uninfected blood, method B
Lane F: red blood cell extraction, uninfected blood, method A
Lane G: *A. centrale*-infected blood, method B
Lane H: *A. centrale*-infected blood, method A
Lane I: Bovine leucocyte DNA
DNA hybridization. DNA was transferred to nitrocellulose and hybridized with a bovine DNA probe labelled with ^{32}P by nick translation (Southern, 1975). Hybridization was for 24 h at 68 °C in a solution containing 6X SSC, Denhardt's solution (5X), 0,05 % SDS and 100 μg/mℓ denatured herring sperm DNA. Bovine DNA, used as the probe, was isolated from leucocytes obtained from an uninfected animal. Post-hybridization washes were performed in 2 changes of 2X SSC containing 0,5 % and 0,1 % SDS respectively. Autoradiography was on Cronex MRF-31 X-ray film at −70 °C for 24 h with a tungstate intensifying screen.

A number of methods have been described which claim to remove most of the white blood cells from parasite-infected blood (Oelshlegel, Sander & Brewer, 1975; Levy & Chow, 1983; Baggaley & Atkinson, 1972). A cellulose column method has been described by Baggaley & Atkinson (1973) for the removal of leucocytes from *Plasmodium*-infected blood. This method resulted in a loss of 20 % of the initial starting red cell material in the retention volume of the column, and no attempt was made to show that the filtrate was totally free of leucocyte DNA. Homewood & Neame (1976) reported that only 1 % of the total volume of treated blood remained after filtration through a CF-12 column. However, in our hands all published protocols still resulted in an unacceptably high level of host cell DNA contamination.

Our results showed that when DNA was extracted by method B, all detectable amounts of bovine leucocyte DNA had been removed from the samples, thus illustrating the effectiveness of the column method in eliminating white blood cells.

The purification protocol described here can be used routinely to isolate pure parasite DNA from infected blood. There is no limitation on the volumes of blood which can be filtered and the method works equally well on small or large volumes. This method insures the availability of uncontaminated DNA, which is an essential prerequisite for studies of the genome of these parasites.

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