Inhibitory effect of syphilitic rabbit serum on DNA synthesis in rabbit cells in vitro

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SUMMARY A previously described toxic factor associated with Treponema pallidum (Nichols) and found in extracts of syphilitic rabbit testes has now also been detected in syphilitic rabbit serum. The toxic factor, which inhibits DNA synthesis in baby rabbit genital organ (BRGO) cells in vitro, is present in rabbit serum up to 30 days after infection with T pallidum.

Introduction

Treponema pallidum (Nichols strain) and extracts of syphilitic rabbit testes inhibit macromolecular (DNA, RNA, and protein) synthesis in rabbit cells in vitro. This finding was one of very few reports of direct toxicity associated with T pallidum. Fitzgerald et al and Oakes et al have also reported mammalian cell toxicity associated with T pallidum. The relevance of these reports is that tissue destruction in early syphilis may be due to direct treponemal toxicity rather than to the host's inflammatory and immune response to the treponeme as was previously assumed.

We report a toxic factor that inhibits the synthesis of DNA in rabbit cells in vitro and is detectable in serum from syphilitic rabbits up to 30 days after infection with T pallidum. Serum from rabbits immunised with heat killed T pallidum was not toxic.

Materials and methods

RABBIT SERUM SAMPLES
Three rabbits were injected with viable T pallidum organisms 50 x 10⁶/testis. The rabbits were bled before infection to provide normal rabbit serum and periodically from five to 150 days thereafter. Control (sham infected) rabbits were injected with heat killed (at 56°C for 30 minutes) T pallidum organisms 50 x 10⁶/testis and bled similarly. Individual samples of serum were stored separately at -70°C until all were ready for experimental use. All serum samples were sterilised by membrane filtration (Millipore 0.45 µm) before coincubation with tissue culture cells.

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COINCUBATION OF BRGO CELLS WITH 3H-THYMIDINE AND SYPHILITIC RABBIT SERUM

About 10^3 BRGO cells were seeded into each well of a 96 well microtitre plate (Sterilin, England) and allowed to attach for 12 hours. This was followed by washing with serum free medium and adding 200 µl syphilitic rabbit serum (20% final concentration). Serum from rabbits injected with heat killed T. pallidum was added to control wells. After aerobic co-incubation for 24 hours, 0.5 μCi 3H-thymidine (20 Ci/mmol, Amersham International, Amersham, England) was added to each well. After incubation for a further 24 hours the BRGO cells were made soluble with 150 µl lysis buffer (0.5 mol/l sodium hydroxide and 0.5% sodium dodecyl sulphate) and washed sequentially with 5% trichloroacetic acid, water, and methanol. The trichloroacetic acid precipitates were collected on to glass fibre filters (Flow Laboratories, Melbourne, Australia) and their radioactivity measured using a Packard Tri-Carb β scintillation counter, with 2,5-diphenyloxazole 5 g/l and 1,4-bis[2-(3-methyl-5-phenyl-oxazolyl)]-benzene 0.4 g/l in toluene as the scintillation fluid.

Results

EFFECT OF SYPHILITIC RABBIT SERUM ON DNA SYNTHESIS IN BRGO CELLS

Serum from rabbits injected with heat killed T. pallidum (5 × 10^5/testis) had no inhibitory effect on DNA synthesis in BRGO cells, while serum taken from rabbits during early syphilis (6 to 30 days after infection) did inhibit DNA synthesis. The figure shows that synthesis was only 40-70% of that using day 0 rabbit serum from the same rabbits. Serum taken later in the disease (40 to 120 days after infection), however, did not appreciably inhibit the BRGO cells. Heat inactivated and non-inactivated serum samples showed similar results, indicating that the toxic factor in the syphilitic rabbit serum was stable to 56° C for 30 minutes (data not shown).

Serum taken from eight other rabbits during peak syphilitic orchitis (11 to 14 days after infection) all inhibited DNA synthesis in BRGO cells by between 64% and 93% (mean 81%), compared with serum from rabbits injected with heat killed or sonicated T. pallidum (data not shown).

Discussion

At the peak of syphilitic orchitis a soluble factor, which is cytostatic for rabbit cells in culture and apparently of treponemal origin, is present in rabbit testes infected with T. pallidum. This toxic substance may play a part in destruction of local tissue in early syphilis. We have now detected a toxic substance in the serum of syphilitic rabbits up to 30 days after infection. This toxin may reasonably be assumed to be of the same origin as the cytotoxins in whole T. pallidum organisms and syphilitic rabbit testes. It may be responsible for some of the systemic effects of disseminated (secondary) syphilis. The effect of this toxic factor on cells of the host's immune system...
Inhibitory effect of syphilitic rabbit serum on DNA synthesis in rabbit cells in vitro has not yet been tested, but the transient immunosuppression seen in experimental and human syphilis may well be mediated by such a mechanism.6-8

Apart from inhibition of DNA synthesis, morphological changes and detachment of BRGO cells were seen after three days' exposure to 10^8 or more treponemes/ml.1 Uninfected BRGO cells were normal in appearance. The relatively low growth ceiling for T pallidum seen in tissue culture9 may be due to inactivation of the mammalian cells by a treponemal toxic factor.

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References